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Growth Through Agricultural Progress

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PRESERVATION OF PLANT VIRUSES IN LIQUID NITROGEN

H. H. McKinney, Lester W. Greeley, and W. A. Clark[1]

Preliminary tests were conducted at the American Type Culture Collection and at the U. S. Plant Industry Station to determine if the six typical labile viruses listed will survive a short period (14 to 21 days) of storage in liquid nitrogen (-196° C). The following viruses were tested:

Barley stripe-mosaic virus (BSMV), type strain, AC-69.
Cucumber mosaic virus (CMV), yellow mosaic strain isolated from AC-10.
Lily-fleck corn virus (LFCV), from F. P. McWhorter, Oregon State University, Corvallis.
Sugarcane mosaic virus (SMV), from E. F. Todd, U. S. D. A., Canal Point, Florida.
Tobacco ringspot virus (TRSV), AC-98.
Tobacco streak virus (TSV), AC-44.

Fresh and/or dehydrated[2] infected leaf tissues were used. Unless stated otherwise, the tissue samples were placed in small glass tubes sealed with heat or with tight fitting stoppers, and on removal from the liquid nitrogen, the tubes were plunged immediately into water and agitated at 37° C for 4 minutes. The stored samples were tested for virus activity at the Plant Industry Station within 2 to 4 hours after removal from the liquid nitrogen. Neither time nor space permitted more than rough activity tests in suitable test plants.

All the viruses were active at the end of the storage period, and possibilities for the long-time storage of certain labile plant viruses appear good. The LFCV, SMV, TRSV, and the TSV were tested in dehydrated tissue. On removal from the liquid nitrogen, one tube of each was plunged into the 37° C water bath, and another tube was allowed to thaw at room temperature. The activity tests revealed no essential differences between rapid and slow thawing. The SMV was limited to a very small amount of infected corn leaf tissue that had been dehydrated near 4° for 41 days before being stored in the liquid nitrogen. The virus was moderately active, but there was insufficient tissue for determination of the activity of the dehydrated tissue before it was stored in liquid nitrogen.

The BSMV and the CMV in fresh and dehydrated leaf tissues were tested with and without a 30% solution of glycerine in the tubes. After removal from the liquid nitrogen, all the tubes were plunged into the 37° water bath for 4 minutes. The fresh and the dehydrated tissues without glycerine were essentially the same in virus activity. The fresh leaf sample of BSMV containing glycerine was inactive. The other samples containing glycerine seemed to be about as active as those without.

CMV in fresh tobacco leaf tissue was stored in liquid nitrogen in a cloth envelope which allowed immediate contact of the tissue with the liquid nitrogen. On removal from the liquid nitrogen, the tissue was allowed to thaw at room temperature. The virus in this sample was very active.

CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, BELTSVILLE, MARYLAND AND AMERICAN TYPE CULTURE COLLECTION, WASHINGTON, D. C.

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[1]Collaborator, and Agricultural Research Technician, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, and Director, American Type Culture Collection, respectively.

[2]McKinney, H. H. Ann. New York Acad. Sci. 56: 615-620. 1953. Dehydration method is described in detail.

2-n-ALKYLMERCAPTO-1, 4, 5, 6-TETRAHYDROPYRIMIDINES, CHEMOTHERAPEUTIC AGENTS FOR PLANT RUSTS[1]

Louis G. Nickell[2, 3], Philip N. Gordon[2], and Alvaro Goenaga[4]

Abstract

Salts of these compounds were effective against rusts of bean, snapdragon, canna, and wheat when applied as foliar sprays. Systemic activity was demonstrated when some of these compounds were tested against rust and mildew of both bean and snapdragon.

2-n-Alkylmercapto-1, 4, 5, 6-tetrahydropyrimidine hydrohalides (AMTP) were prepared by reaction of an n-alkyl halide with hexahydropyrimidine-2-thione (1).

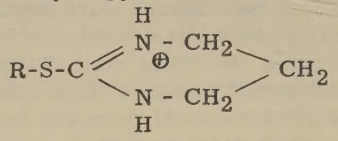

FIGURE 1.

When tested in vitro against an array of phytopathogenic fungi, the n-alkyl homologs from the methyl through the hexyl showed no antifungal activity at levels of 1000 mcg/ml when incorporated into an agar nutrient medium. Slight activity against four of the pathogens was shown by the heptyl AMTP. The activities of the higher members are shown in Table 1. The even-numbered carbon chain homologs show a wider spectrum of activity and greater potency than do the odd-numbered members.

When tested against a variety of plant pathogens in vivo, two differences are noticed. First, the lower members (methyl through hexyl) show progressively increasing activity against early blight of tomatoes. Second, both odd and even carbon chain homologs are quite active (Table 2). The most interesting activity was that shown against bean rust, where all members from 8 through 14 carbons gave complete control at 80 ppm.

Some alkyl-substituted tetrahydropyrimidines were reported by Rader, et al. (2) to have fungistatic activity against late blight of tomatoes and late blight of celery. These compounds showed no control of powdery mildew and only moderate control of bean rust, in contrast to the AMTP series to which they are distantly related.

In other tests, the 8 and 14 carbon AMTP compounds which encompass the extremes of activity were further evaluated and showed good activity against the rusts of canna, wheat, and snapdragon.

The most extensive testing carried out with these two homologs was an evaluation, under practical conditions, in a commercial greenhouse with second-year snapdragon plants heavily infested with rust, including stem cankers from the previous year. Three similar blocks consisting of approximately 50 plants each were selected for the tests. A total of nine applications of the n-octyl and n-tetradecyl AMPT compounds at 100 ppm were made at weekly intervals from November to January. Triton X-114 at 125 ppm was used as the wetting agent. Both stem and leaf infections were very heavy when the test began. Data on infection at the end of the test period are summarized in Table 3. The superiority of the n-octyl AMTP was clearly demonstrated.

During this series of tests, a heavy outbreak of powdery mildew occurred in the greenhouse. Excellent control of this infection was noted in the blocks treated with both of these compounds. A separate test set up specifically against the mildew confirmed these results.

Subsequent to these controlled experiments, the entire greenhouse was treated with one of the AMTP preparations. As a result both rust and mildew were essentially eliminated, permitting normal development through flowering of the entire crop in the greenhouse.

Systemic activity of the n-octyl AMTP completely prevented mildew and rust infections in

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[1]Cooperative investigations of Research Laboratories, Chas. Pfizer & Co., Inc., Groton, Connecticut and the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York.

[2]Chas. Pfizer & Co., Inc.

[3]Present address: Experiment Station, Hawaiian Sugar Planters' Association, Honolulu, Hawaii.

[4]Boyce Thompson Institute.

Table 1. Effect of various members of the AMTP series against a group of phytopathogenic fungi as shown by incorporation in nutrient agar.

Sidechain length	Alternaria solani	Botrytis allii	Septoria nodorum	Endothia parasitica	Fusarium oxysporum	Ceratostomella ulmi	Sclerotinia fructicola	Verticillium albo-atrum	Helminthosporium victoriae	Glomerella cingulata	Phoma betae	Fusarium lycopersici var. bulbigenum	Diplodia zeae	Rhizoctonia solani	Pythium debaryanum	Cercospora musae
n-Heptyl-	1	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0
n-Octyl-	1	1	2	1	1	2	1	1	1	1	1	1	2	1	1	0
n-Nonyl-	2	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0
n-Decyl-	2	1	4	0	0	2	2	1	1	2	2	2	3	2	2	3
n-Hendecyl-	2	2	0	0	2	0	0	0	2	0	0	0	0	0	2	0
n-Hendec-10-enyl-	2	3	0	0	1	0	0	0	0	0	0	0	0	0	1	0
n-Dodecyl-	2-	0	2	0	2	2	3	0	2	0	2	2	3	1	2	4-
n-Tridecyl-	3	3	0	0	2	0	0	0	0	0	0	0	0	0	1	0
n-Tetradecyl-	2	0	2	1	1	2	0	2-	2-	1	0	1	4-	0	1	4
n-Hexadecyl-	0	0	0	1	1	2	1	0	0	0	0	0	4	0	1	3
n-Octadecyl-	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	2

Legend: 0 - inactive, or active $>$1000 ppm; 1 - active at 1000 ppm; 2 - active at 100 ppm; 3 - active at 10 ppm; 4 - active at 1 ppm; - partial inhibition.

Table 2. Effects of AMTP compounds at 80 ppm on several plant diseases in greenhouse tests when applied as foliar sprays.

R = (see Fig. 1)	Tomato		Bean	
	early blight[a]	late blight[b]	mildew[b]	rust[a]
n-Hexyl-	88	1	3	88
n-Octyl-	87	1-2	3	100
n-Nonyl-	87	0-1	2-3	100
n-Decyl-	88		0-1	100
n-Undecyl-	94	1	3	100
n-$\triangle^{10,11}$ Undecylenyl	90	1	3	100
n-Dodecyl-	87	2	2-3	100
n-Tridecyl-	71	2-3	3	100
n-Tetradecyl-	82		3	100
n-Hexadecyl-	77		3	76
n-Octadecyl-	63		3	0

[a] % control.

[b] Arbitrary figures with 0 representing excellent control and 3 no control.

Table 3. Effectiveness of AMTP analogs in controlling rust on severely infected snapdragon plants.

Compound	No. plants infected / No. plants inspected: stems	foliage	Total no leaves infected	% control
Check	19/20	20/20	225	--
n-Octyl	8/20	5/20	10	95
n-Tetradecyl-	9/20	18/20	82	64

bean plants treated 3 days prior to inoculation, by the application of 100 mgm of material to the soil per 4-inch pot. Under these same conditions the n-tetradecyl AMTP gave 67% control. Both compounds showed systemic action against Fusarium wilt of tomatoes when the plants were pre-treated in nutrient solutions containing the test compounds prior to transplanting to soil and inoculating.

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RESEARCH LABORATORIES, CHAS. PFIZER & CO., INC., GROTON, CONNECTICUT AND BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC., YONKERS, NEW YORK

APPLE SCAB 1. EFFECT OF ONE APPLICATION OF FUNGICIDES ON LEAF LESIONS ON PREVIOUSLY UNSPRAYED TREES[1]

J. J. Albert and J. W. Heuberger[2]

Summary

Three field experiments were conducted in 1960, on apple trees previously unsprayed that season, to determine the possible effects on apple scab leaf lesions of a single application of fungicide materials in common use. Data were obtained over a period of approximately 1 month on (a) lesion development, (b) spore germination, and (c) viability of the organism in the subcuticular area of the leaf. The data showed (a) that all materials arrested subsequent increase in size of the lesion for at least 8 days, (b) that all materials depressed spore germination for varying periods of time, and (c) that no material killed the organism within the leaf. Dodine (Cyprex) appeared to have the most pronounced effects.

INTRODUCTION

Each year numerous field experiments are conducted the world over to evaluate various fungicides for control of the apple scab disease. The evaluation is based on incidence and severity of scab on the leaves and fruit. Little attention is paid to how the fungicides bring about control. Hamilton (3) discussed this problem in 1931 and Powell (5) has recently reiterated it.

For many years the terms "eradication" and/or "burning-out" have been used in cases where growers have sprayed liquid lime sulfur and/or organic mercury fungicides on incipient and visible established scab lesions in an attempt to control the disease. The term "kick-back" has been used in recent years for those cases where growers applied a fungicide from 24 to 72 hours after an infection period had begun in an attempt to destroy the germinating organism. What is the action of the fungicide in these situations? Is the organism destroyed? Are the spores on the lesions killed or simply inhibited for as long as the fungicide remains active? Is spore production prevented? And, in the case of visible established lesions, is the organism destroyed below the cuticle? Few answers are available. So far as the writers are aware, no one has yet shown that the scab organism has been destroyed within the subcuticular area of the leaf or fruit where visible established lesions were present. Hamilton (3) reported in 1931 that Bordeaux mixture (4-4-100)[3], lime sulfur-aluminum sulfate mixture, lime sulfur (1-40)[4] plus lead arsenate (1-50)[5] may entirely kill the fungus in the lesions; however, no data were presented. Some data are available, however, on the effect of the fungicides on inhibition of spore germination, on spore production, and on the lesion.

Hamilton and Keitt (4), in 1928, stated that liquid lime sulfur (1-40)[4] plus lead arsenate (1-50)[5] applied to scabbed leaves in the orchard prevented the germination of spores which were present and sharply inhibited production of spores from the treated lesions during a period of 9 weeks.

Weaver (7), in 1958, studied the effect of dodine (Cyprex 65W) on established apple scab leaf lesions on the Red Delicious and Stayman varieties. Dodine was applied from the period of the Second Cover Spray (May 27-28) through the Seventh Cover Spray (August 5) and compared with captan 50W (2-100) used in the same schedule. The concentration of dodine was 1 1/2-100 on Stayman and 2-100 on Red Delicious at the first application. This was reduced in succeeding applications until a concentration of 1/2-100 was used from the Fourth Cover through the Seventh Cover. He found that severely scabbed leaves on the Red Delicious variety turned yellow and fell after dodine (2-100) was applied, there was no defoliation on Stayman. He stated that on June 10, 13 to 14 days after the first application and 6 to 8 days after the second, "the scab spots on dodine-sprayed trees had turned a reddish to purple color and appeared inactive. The mycelium and conidia appeared to be eradicated while scab lesions on captan-sprayed trees re-

[1]Published as Miscellaneous Paper No. 402 with the approval of the Director of the Delaware Agricultural Experiment Station. Contribution No. 142 of the Department of Plant Pathology.

[2]Respectively: former Graduate Assistant, University of Delaware, now Graduate Assistant, Pennsylvania State University Fruit Research Laboratory, Arendtsville, Pa.; and, Professor, Department of Plant Pathology, University of Delaware, Newark.

[3]Four pounds copper sulfate, 4 pounds spray lime, 100 gallons water.

[4]One gallon liquid lime sulfur, 40 gallons water.

[5]One pound lead arsenate, 50 gallons water.

mained black and active."

Weaver (8), in 1959, conducted a second experiment on the Stayman variety with dodine (1/2-100) where it was applied from May 20 for the balance of the season to foliage with well-established leaf lesions. Captan (2-100) was used for comparison. Seven sprays were applied from May 23 to August 14, 1959. He observed that "the eradicative effects of Cyprex were observed on June 10; the drying-up of scab spots was not as complete as noticed on the Red Delicious variety." Weaver concluded, after 2 years' work, that "although Cyprex (dodine) has some eradicative properties, it will not eradicate established scab lesions on mature apple leaves."

Lewis[6], in 1959, reported to the Cumberland-Shenandoah Fruit Workers Conference on his research on the effect of fungicides on the inactivation of visible scab lesions. Trees in an abandoned orchard were sprayed on June 15, 1959 with captan (2-100), dodine (1-100), and dodine (1/2-100). In addition, individual scab lesions on unsprayed trees were marked for identification and then sprayed with an atomizer containing either captan or dodine. The number of spores present per square millimeter of lesion and the percentage spore germination on glass slides were determined at 2, 4, 9, 16, 23, and 25 days after spraying. His data showed the following: (a) 2 days after spraying, the number of spores on the captan-sprayed lesions was 208 per square millimeter compared with 1139 on the unsprayed lesions but 4 days after spraying the captan plots and the unsprayed plots were not significantly different in spore numbers; (b) with dodine, the number of spores was about 800 two days after spraying followed by a nearly steady decline up to the twenty-third day, after which there was an upturn in spore numbers; (c) no spore germination was obtained from the captan-sprayed plots until the twenty-third day and from the dodine-sprayed plots until the twenty-fifth day; and, (d) no eradication occurred in the sense of killing the scab spot and the leaf tissue beneath it. Lewis concluded that dodine has a more pronounced effect than captan on spore numbers and germination.

Szkolnik and Hamilton (6), in 1960, in a brief review of their work with dodine, stated that dodine had eradicative properties in the sense of killing or inactivating the disease fungus in the plant after it had already started an infection. In addition, they pointed out that dodine also had "local" systemic activity.

Powell (5), in 1960, reported on the inhibitory effects of certain fungicides on apple scab conidia. He determined the effect on the germination of the conidia for periods of 1, 5, 10, and 21 days after leaves, attached to the trees, were dipped in various concentrations (2400, 1200, 600, 300, 150 ppm) of the fungicides. He found that dodine, captan, Phaltan, and Dyrene checked germination at 2400 ppm for a 21-day period. At lower concentrations, however, a significant spore recovery occurred after 10 days. Of the four materials, dodine exhibited a greater residual toxicity as the time intervals increased. Other materials used (Niacide M, dichlone, and zineb) were not as effective. Captan and Phaltan, at 2400 ppm, interfered with the normal release of the conidia.

In preliminary work in 1958 and 1959 on the effect of liquid lime sulfur and organic mercury (Tag 331) on previously unsprayed scab leaf lesions, the junior author found that both materials inhibited spore germination for at least three weeks and, in addition, reduced the number of spores present on the lesions as compared with the unsprayed lesions.

During 1960, as the start of a long-range study on the effects of fungicides on established apple scab leaf lesions, the authors conducted three experiments to determine the effect of one application of the fungicides in common use for apple scab control on (a) spore germination, (b) increase in size of the lesion, and (c) the organism within the leaf tissue; no attempt was made to determine spore numbers. Two preliminary reports (1, 2) have been made of this work.

MATERIALS AND METHODS

The fungicides[7] and combinations thereof tested were those in common use in the Middle Atlantic States for control of scab. In some cases a material was used in dosage series.

As enough scab was not present in Delaware the first part of the season, the first two tests were conducted in cooperation with Dr. A. B. Groves[8], Winchester, Virginia, on the York variety; the third test was carried out on the Rome variety at the Experimental Station of the Hercules Powder Company[8], Wilmington, Delaware. At both locations the trees used had had no previous application of fungicides in 1960. Due to time limitations, it was not possible to test

[6]Personal communication from F. H. Lewis to the junior author, dated December 6, 1960.

[7]For the common name, trade name, and active ingredient (%) of the materials used, refer to Results of 1960 Fungicide-Nematocide Tests published by the American Phytopathological Society.

[8]The authors acknowledge their indebtedness to Dr. A. B. Groves and the Hercules Powder Company for their complete cooperation that made the research possible.

all materials and concentrations thereof in any one test. Dodine (Cyprex) and captan were used in all three tests, and phenylmercuric acetate (Tag 331), Tag 331 +glyodin, and N-trichloromethyl thiophthalimide (Phaltan) were used in two tests.

The same procedure was followed in all tests. The number of leaves required in each test were tagged the day before the fungicides were applied. These had from one to three lesions. Individual trees were then sprayed once to the run-off point, using a 4-nozzle broom and 300 psi pressure in the first two tests and a hand gun and 450 psi pressure in the third. Immediately after spraying, the lesions were measured in two diameters on the leaves tagged for that purpose (one lesion per leaf). Then, at stipulated intervals after spraying, the following three things were done: (a) the lesions were measured; (b) tagged leaves (1 to 3 lesions per leaf) were brought to the laboratory and the germination of the spores determined, using the leaf disc-depression slide technique described by Powell (5); and, (c) isolations were made of the scab organism present within the subcuticular area of the lesion, using potato-dextrose agar as the growth medium. The surface of the leaf was treated with a weak disinfectant, the cuticle removed, and a piece of tissue removed with a sterile lance and plated.

RESULTS

The complete data from the first test are presented in Tables 1 and 2, from the second in Tables 3 and 4, and from the third in Tables 5 and 6. A brief summary of the data in all tests, under conditions of one spray application, follows:

(a). Cultures of Venturia inaequalis were obtained from lesions receiving all treatments at all dates.

(b). Dodine (1-100) prevented further development of visible leaf lesions for 22 days. Tag 331 (8 oz.-100), dodine (3/4-100), and Phaltan (2-100) prevented development for 15 days. In general, the other materials prevented development for 8 days.

(c). The most effective materials for the prevention of spore germination, in descending order, were: dodine (1-100), Tag 331 (8 oz.-100), liquid lime sulfur (6 qts.-100), dodine (3/4-100), Tag 331 (4 oz.-100), Tag 331 plus glyodin (4 oz.+24 oz.-100), Phaltan (2-100), captan (2-100), and dodine (1/2-100). Materials that prevented spore germination the longest period of time (23 days) were dodine (1-100), Tag 331 (8 oz.-100) and liquid lime sulfur (6 qts.-100).

(d). In general, as the concentration of a material increased, its effectiveness increased.

(e). The materials that prevented spore germination for the longest period of time (23 days) also arrested visible lesion development the longest, except for liquid lime sulfur (6 qts.-100).

(f). Captan (2-100) and Phaltan (2-100) decreased the release of conidia from the conidiophores on the leaf lesions. The addition of lead arsenate (1 1/2-100) seemed to overcome this effect.

Table 1. Venturia inaequalis: Spore germination in the first apple scab test, Winchester, Virginia, 1960.

Material[a] and concentration	% germination days after spraying			
	5	9	16	23
Unsprayed	60	40	93	56
Dodine, 1/4-100	0	11	32	41
do. 1/2-100	0	0	17	23
do. 1-100	0	0	0	0
Captan, 1-100	0	3	29	33
do. 2-100	0	0	2	9
Tag 331, 4 oz.-100	0	0	3	5
Tag 331, 8 oz.-100	0	0	0	0
Tag 331 +glyodin, 4 oz.-24 oz.-100	0	4	3	2
Zineb, 2-100	2	20	72	83
Lead arsenate +lime, 3+3-100	10	25	51	53
Liquid lime sulfur, 3 qts.-100	1	7	64	71
Liquid lime sulfur, 6 qts.-100	0	0	0	0

[a]Sprayed on June 13-14, 1960. Pounds per 100 gallons water except where otherwise indicated.

Table 2. Venturia inaequalis: Lesion development in the first apple scab test, Winchester, Virginia, 1960.

Material[a] and concentration	Avg. size (mm) of lesion when sprayed	Avg. size (mm) of lesion days after spraying[b] 8	15	22	32	Avg. increase in size (mm) in 32 days
Unsprayed	4.6	5.0	5.6	6.2	6.6	2.0
Dodine, 1/4-100	4.4	5.1	6.0	6.8	7.4	3.0
do. 1/2-100	3.5	3.5	4.3	5.3	5.8	2.3
do. 1-100	5.2	5.2	5.2	5.2	5.6	0.4
Captan, 1-100	5.4	5.4	6.2	6.6	7.4	2.0
Captan, 2-100	5.4	5.4	5.8	6.6	7.2	1.8
Tag 331, 4 oz.-100	5.4	5.4	5.6	6.6	7.8	2.4
Tag 331, 8 oz.-100	4.6	4.6	4.6	5.0	6.0	1.4
Tag 331+glyodin, 4 oz.+24 oz.-100	5.6	6.2	6.6	7.4	7.4	1.8
Zineb, 2-100	5.2	5.2	5.8	7.4	9.2	4.0
Lead arsenate+lime, 3+3-100	5.2	5.2	6.2	6.8	7.4	2.2
Liquid lime sulfur, 3 qts.-100	5.4	5.4	6.4	6.6	8.0	2.6
Liquid lime sulfur, 6 qts.-100	5.8	5.8	6.0	7.0	7.2	1.4

[a]Sprayed June 13-14. Pounds per 100 gallons water except where otherwise indicated.
[b]Based on the scoring of 1 lesion on each of 5 leaves.

Table 3. Venturia inaequalis: Spore germination in the second apple scab test, Winchester, Virginia, 1960.

Material[a] and concentration	% germination days after spraying 2	9	16	23
Unsprayed	17	43	37	31
Dodine, 1/2-100	0	0	2	3
do. 3/4-100	0	0	0	1
do. 1-100	0	0	0	0
Captan, 2-100	0	0	2	1
Captan+zineb, 1+1-100	0	2	3	5
Captan+lead arsenate, 2+1 1/2-100	0	0	0	1
Lead arsenate+lime, 3+3-100	0	21	27	23
Phaltan, 2-100	0	0	1	0
Maneb, 2-100	0	1	0	11
Tag 331, 4 oz.-100	0	0	3	2
Tag 331+glyodin, 4 oz.+24 oz.-100	0	0	3	3
Glyodin, 24 oz.-100	0	0	4	3

[a]Sprayed on July 18, 1960. Pounds per 100 gallons water except where otherwise indicated.

Table 4. Venturia inaequalis: Lesion development in the second apple scab test, Winchester, Virginia, 1960.

Material[a] and concentration	Avg. size (mm) of lesion when sprayed	Avg. size (mm) of lesion days after spraying[b] 8	15	22	32	Avg. increase in size (mm) in 32 days
Unsprayed	4.0	4.4	5.4	6.2	7.2	3.2
Dodine, 1/2-100	4.4	4.4	4.8	5.0	6.6	2.2
do. 3/4-100	4.2	4.2	4.2	4.4	5.0	0.8
do. 1-100	4.4	4.4	4.4	4.4	4.6	0.2
Captan, 2-100	4.0	4.0	5.0	5.2	6.0	2.0
Captan+zineb, 1+1-100	4.4	4.4	5.4	6.2	7.0	2.6
Captan+lead arsenate, 2+1 1/2-100	3.4	3.4	4.0	4.0	4.6	1.2
Lead arsenate+lime, 3+3-100	4.8	4.8	5.8	5.8	6.2	1.4
Phaltan, 2-100	4.2	4.2	4.6	4.6	5.0	0.8
Maneb, 2-100	3.8	3.8	4.8	4.8	6.0	2.2
Tag 331, 4 oz.-100	4.2	4.2	5.0	5.2	6.6	2.4
Tag 331+glyodin, 4 oz.+24 oz.-100	3.2	3.2	4.0	4.4	5.2	2.0
Glyodin, 24 oz.-100	5.0	5.0	5.8	6.8	7.4	2.4

[a]Sprayed July 18, 1960. Pounds per 100 gallons water except where otherwise indicated.
[b]Based on the scoring of 1 lesion on each of 5 leaves.

Table 5. Venturia inaequalis: Spore germination in the third apple scab test, Hercules Experiment Station, Wilmington, Delaware, 1960.

Material[a] and concentration	% germination days after spraying 2	9	16	19	20	21	22	23	24	25
Unsprayed	93	91	94	--	--	--	--	98	--	--
Dodine, 1/2-100	0	2	35	--	--	--	--	65	--	--
do. 1-100	0	0	0	1	3	3	7	17	23	51
Captan, 1-100	7	59	93	--	--	--	--	94	--	--
Captan, 2-100	0	21	79	--	--	--	--	85	--	--
Phaltan, 1-100	4	56	73	--	--	--	--	93	--	--
Phaltan, 2-100	0	13	39	--	--	--	--	71	--	--
Lead arsenate+lime, 3+3-100	17	34	91	--	--	--	--	93	--	--
Captan+zineb, 1+1-100	16	94	93	--	--	--	--	97	--	--
Captan+zineb+lead arsenate, 1+1+1 1/2-100	14	87	92	--	--	--	--	91	--	--
Captan+lead arsenate, 1+1 1/2-100	13	76	83	--	--	--	--	92	--	--
Phaltan+lead arsenate, 1+1 1/2-100	11	65	75	--	--	--	--	89	--	--

[a]Sprayed on September 29, 1960. Pounds per 100 gallons water except where otherwise indicated.

Table 6. Venturia inaequalis: Lesion development in the third apple scab test, Hercules Experiment Station, Wilmington, Delaware, 1960.

Material[a] and concentration	Avg. size (mm) of lesion when sprayed	Avg. size (mm) of lesion days after spraying[b] 8	15	22	32	Avg. increase in size (mm) in 32 days
Unsprayed	3.0	3.8	4.6	5.6	7.6	4.6
Dodine, 1/2-100	3.8	3.8	4.4	4.8	6.4	2.6
do. 1-100	5.0	5.0	5.0	5.0	6.0	1.0
Captan, 1-100	4.6	4.6	5.1	6.2	7.8	3.2
Captan, 2-100	3.2	3.2	4.0	5.2	6.0	2.8
Phaltan, 1-100	4.6	4.6	4.8	6.0	7.6	3.0
Phaltan, 2-100	3.4	3.4	3.4	4.8	5.8	2.4
Lead arsenate+lime, 3+3-100	4.6	4.6	4.8	6.4	7.6	3.0
Captan+zineb, 1+1-100	3.2	3.2	4.2	4.8	6.2	3.0
Captan+zineb+lead arsenate, 1+1+1 1/2-100	4.0	4.0	6.0	6.0	7.4	3.4
Captan+lead arsenate, 1+1 1/2-100	3.6	3.6	4.4	5.4	7.6	4.0
Phaltan+lead arsenate, 1+1 1/2-100	3.8	3.8	4.6	5.6	7.2	3.4

[a]Sprayed September 29, 1960. Pounds per 100 gallons water except where otherwise indicated.
[b]Based on the scoring of 1 lesion on each of 5 leaves.

DISCUSSION

From the data obtained it is apparent that dodine (1-100) was the best all-around material in the test. Even at a concentration of 3/4-100 it still was outstanding. When used at a concentration of 1/2-100, now fairly standard in orchard practice, it was similar to captan (2-100) and Phaltan (2-100) in its effects. It was interesting to note in the data that dodine was more than twice as effective at 1-100 than at 1/2-100.

The data obtained with dodine on spore germination under conditions of one spray application agree with those obtained by Powell (5) and by Lewis[6]; those on lesion development help explain, in part, the observations made by Weaver (7, 8) on the effect of dodine on lesions under conditions of repeated applications. Also, the data are in agreement with those of Powell (5) on the influence of captan and Phaltan, 2-100, on retardation of release of conidia.

The data show that under conditions of one spray application no eradication of the fungus occurred. The possible effect of serial applications needs to be determined.

The germination of conidia from unsprayed lesions was very poor (low) in the second Winchester test. This skewed the data considerably. However, where the same materials and concentrations were used in both the first and second Winchester tests, the data are in line as to their relative effectiveness.

In the work reported herein, the age of the lesions and the spores thereon was unknown. In the first and second Winchester tests, it was assumed that the lesions used had developed from ascosporic inoculum; in the third test, the lesions used had developed from conidial inoculum. However, this did not affect the relative performance of the materials from test to test.

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DOWNY MILDEW ON SMALL GRAINS AND TWO OTHER GRASSES IN MISSISSIPPI, 1961[1]

P. G. Rothman and D. H. Bowman[2]

Downy mildew, incited by Sclerophthora macrospora (Sacc.) Thirum., Shaw & Naras., was found at Stoneville, Mississippi in the spring of 1961 on three hosts previously unreported from that State. These were rye (Secale cereale), common ryegrass (Lolium multiflorum), and little barley (Hordeum pusillum).

Recent reports (1, 2, 5, 6, 8) show an ever-widening group of hosts for S. macrospora. While the fungus is not especially destructive in the United States, it is a potential menace to small grains through its restricted and sporadic appearances. The ability of downy mildew to remain dormant for extended periods and the increasing list of susceptible grass weeds which inhabit the fields present serious problems for control of the fungus.

Downy mildew attacked all the small grains growing at Stoneville, Mississippi during 1960-1961 (Fig. 1). The first report of S. macrospora on oats in Mississippi was made by Miles (3). Later, Miles and Epps (4) published an account of the disease on oats and wheat in this State. Wells (8) reported the first occurrence of downy mildew on barley in Mississippi in 1956. This note is the first report of S. macrospora parasitizing rye in Mississippi. The disease has been reported previously in Arkansas by Dale and Templeton (1) in rye purity-trial plantings.

The downy mildew-infected plants of Rogers barley were restricted to a strip along a low south end of the field. The diseased rye plants were scattered along a low west border of a foundation seed field of Explorer rye. The field had not been planted to small grain for 5 years. However, downy mildew had occurred in an adjacent field planted to oats 2 years previously. Drainage water undoubtedly played a part in spreading the organism to the rye field.

Infected oat plants were found scattered in a foundation seed field of Delta Red 88 (Avena byzantina). They appeared to occur in depressions where water stood for extended periods during the early growing season. The field had been planted to soybeans or cotton for the previous 13 years.

In this same field, a small patch of Bledsoe wheat and two grasses occurring as weeds, common ryegrass (Lolium multiflorum) (Fig. 2) and little barley (Hordeum pusillum (Fig. 3), also were infected with downy mildew. The "Index of Plant Diseases in the United States" (7) does not list S. macrospora as reported previously on any noncultivated species of Hordeum or on any Lolium species. However, the fungus has been reported on L. perenne and L. temulentum in other countries (4).

Symptoms, common to all reported species, were excessive tillering, shortening of the internodes and flower pedicel, abnormal thickening and twisting of the leaf tissue, and mass proliferation of vegetative shoots from the floral bracts. Embedded oospores of the fungus could be observed readily in the plant tissue at maturity.

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[1]Cooperative investigations, Crops Research Division, Agricultural Research Service, United States Department of Agriculture and Mississippi Agricultural Experiment Station. Journal Paper (New Series) No. 959.

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FIGURE 1. Panicles of small grains infected with S. macrospora: A -- oats var. Delta Red 88. B -- wheat var. Bledsoe. C -- rye var. Explorer. D -- barley var. Rogers.

FIGURE 2. Panicles of ryegrass infected by S. macrospora (A, B, C) and a healthy panicle (D).

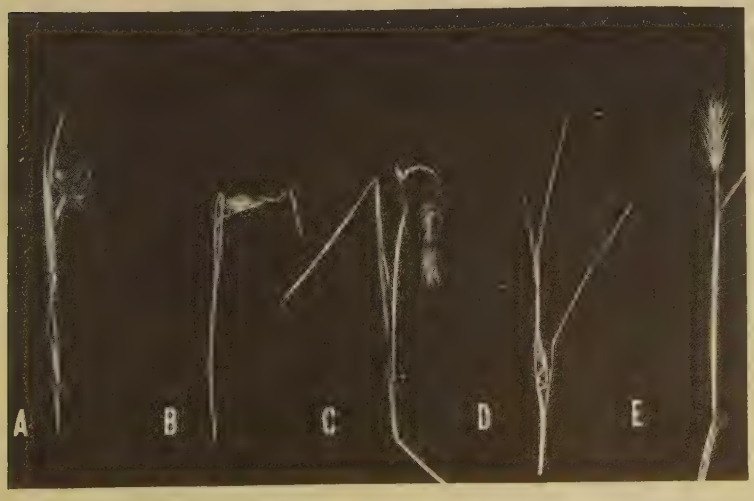

FIGURE 3. Panicles of little barley showing symptoms of S. macrospora (A, B, C, D) and a healthy panicle (E).

CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, STONEVILLE, MISSISSIPPI IN COOPERATION WITH THE MISSISSIPPI AGRICULTURAL EXPERIMENT STATION

TECHNIQUES FOR HANDLING THRIPS IN TRANSMISSION EXPERIMENTS WITH THE TOMATO SPOTTED WILT VIRUS[1]

K. Sakimura

Summary

Techniques used for handling thrips vectors in transmission experiments with the tomato spotted wilt virus in the writer's various projects, including the two recent ones with Frankliniella occidentalis (Pergande) and Thrips tabaci Lind. in California, and with F. fusca (Hinds) in New Jersey, are described in full for the first time. This paper is an attempt to make the techniques developed for this rather unfamiliar subject matter available to other workers. The descriptions and remarks cover selection of experimental plant species, examining and handling thrips, various types of cage and rearing methods, and acquisition and test feedings.

The rather slow accumulation of our knowledge on interrelationships between thrips vectors and the tomato spotted wilt virus has been thought, in some quarters, to be due to the unfamiliarity of the workers with the techniques of handling thrips, which may require some specific manipulations. This situation must have been aggravated by published descriptions of those techniques, too brief to be readily reproduced by other workers. An attempt is made herewith to correct this situation. Specifically, techniques that the writer used in the various projects, including the two recent ones conducted at Berkeley, California in 1956 (7, 8)[2] and at Princeton, New Jersey in 1948 (9) are to be described in full for the first time. The techniques are, however, believed to be in need of further improvements and refinements.

In classical experiments conducted as early as 1927 and thereafter, Samuel and his associates (3, 10) used a "small tube-spring clip" cage for restricted feeding and a lamp-globe cage for mass rearing of thrips. The insects were handled with a camel's hair brush in a specially constructed small transfer room. Linford (2) used a jar or celluloid-covered cage for feeding, and a specially designed aspirator and small transfer chamber for handling insects. Smith (11) used a "micro-cage" mounted on wooden pot labels for restricted feeding. A new technique was introduced in 1948 by Wright, et al. (12, 13) who demonstrated a sticky ring barrier for acquisition feeding on floating detached leaves and a micro-sandwich cage made of foam rubber. The writer has used various types of cylinder cages, a micro-sandwich cage made of felt, and a simple aspirator since his first experiments in 1937 (6).

PLANT SPECIES USED FOR REARING AND TRANSMISSION TESTS

Different thrips species may vary in their host preference and feeding habits. In transmission experiments with a particular thrips species, a proper selection of plant species should be made for rearing as well as for acquisition and test feedings. Often the preferred natural host plant may not necessarily be the most suitable one for experimentation. Some qualifications needed for good rearing plants are good tolerance for thrips feeding and morphological fitness for easy recovery of thrips. Flowers are usually not suitable. For flower-feeding thrips, however, some plant species on which they can thrive on foliage or fruit can be utilized. Good source plants of the virus should provide complete nutrition for the larvae, non-necrotic reaction from infection, and thorough systemic infection with high virus titer for a prolonged period. Good test plants for the virus require high susceptibility to infection and manifestation of conspicuous symptoms. For a particular thrips species some plant species may qualify for all three purposes, others for one or two only.

Emilia sonchifolia was found by the writer to be satisfactory as a rearing, source, and test plant for Thrips tabaci Lind., T. nigropilosus Uzel, Frankliniella sulphurea Schmutz, F. fusca (Hind.), Chaetanaphothrips orchidii (Moulton), Hercinothrips femoralis (Reuter), Kurtomathrips morrilli Moulton, and Parthenothrips dracaenae (Heeger). E. sonchifolia and its closely allied species are pan-tropic composite weeds in the tropical Americas including Florida, tropical Africa, and Indo-Malay-Oceania region including southern China and south-

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ern Japan, and northern Australia. It grows readily in greenhouses with little attention. The virus in Emilia, however, is neither concentrated nor thoroughly systemic.

Frankliniella occidentalis (Pergande), which was used in the Berkeley project, is essentially a flower feeder. As the food acceptance test (Table 1) shows, Emilia and cabbage (Brassica oleracea capitata) were not fully satisfactory for rearing or acquisition feeding. However, following the earlier experience of Bryan and Smith (1), bean pods (Phaseolus vulgaris) were adopted for rearing. For acquisition feeding, however, the larvae were allowed to feed on the Emilia source plants only during their early life, and returned to bean pods during the last 2 feeding days (Fig. 1).

Table 1. Food acceptance of Frankliniella occidentalis.

Plant	Adult feeds	Lays eggs	Larva feeds	Completes immature stages
Emilia, leaves	less freely	occasionally	freely	impossible
Bean, pods	freely	abundantly	freely	freely
Cabbage, midribs	freely	sparingly	freely	occasionally

EXAMINING AND HANDLING THRIPS

Establishing homogeneous colonies of properly identified thrips species is essential prior to the start of transmission experiments. Retaining a few sample specimens and identifying them after the termination of experiments is undesirable. Since thrips taxonomy is highly technical, specialists should be consulted for determinations of the species.

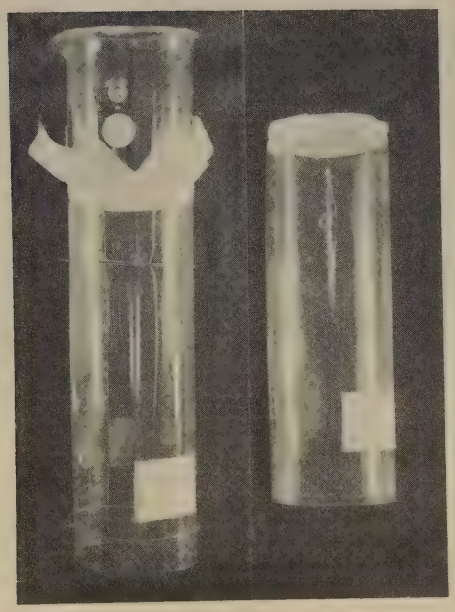
Critical examinations for identity of the live specimens are often required during the course of experiments, for instance, when new colonies are established or prior to transferring insects for test feeding and particularly of field insects in epidemiology studies. For this purpose, CO_2 gas is advantageously utilized for anesthetizing thrips. Thrips confined in a small cloth-covered vial are placed on the bottom of a Büchner funnel that is connected to a flow of gas; inactivated thrips, still in the vial, are examined under a microscope. In one test, inactivation for 2, 5, 10, and 20 minutes resulted after exposure to the gas for 4, 12, 28, and 42 minutes, respectively. Another simple method for inactivating thrips is to expose them to low temperature in a deep freezer for a very short time, a method that is particularly useful for cursory sorting of the specimens collected in the field. Still another method is trapping thrips at the bottom of a vial by applying gentle pressure upon a soft ball of cotton; the specimens can then be satisfactorily examined under a microscope.

The basic principle for handling thrips is as follows: First, the insects are shaken off from the plants over black or white paper or cloth from which they are then picked up. At room temperature, adults are too active to handle safely with a brush. They may, however, be collected with a small simple aspirator which fits a small vial (15 mm x 25 or 45 mm). To transfer thrips into a cage, the aspirator head is first removed from the vial, and then the insects are dropped by tapping the bottom of the vial, held upside-down, with the finger tip. Adults can be confined without food up to 30 minutes in such vials. Larvae are sluggish in moving and very fragile. Transfers are safely effected by picking up the thrips with a fine-pointed camel's hair brush (size 00 or 000) which is slightly moistened by saliva. As thrips are all minute in size, a magnifocusser, low power (10 times) binocular fastened on the forehead, is very useful for handling thrips in any phase of transmission experiments.

CAGES FOR CONFINING THRIPS ON POTTED PLANTS

Whether for confining a colony or single insect, the basic requirements for constructing the cages are: 1) thorough sealing off without any opening larger than 400 mesh (.0025 inches), otherwise thrips frequently escape; 2) avoiding heavy dew condensation on the inner surface of cage; 3) designing for easy collecting and releasing of insects. For covering cages, broadcloth, raw silk or sheer dacron are satisfactory, whereas batiste, organdy or sheer nylon are too porous to confine thrips. Ample cloth-covered windows on the wall of the cage, sparse plant growth within, subsurface irrigation, and protection from sudden temperature changes may all be helpful for avoiding heavy dew condensation within the cages.

For mass rearing of thrips, large metal- or wooden-framed cages, covered partly with plastic sheeting and cloth, are satisfactory. For confining an individual plant for acquisition or test feeding, lantern chimney, glass or plastic cylinder cages are equally satisfactory. A small plastic cylinder cage (Fig. 1), easily and inexpensively constructed, was particularly



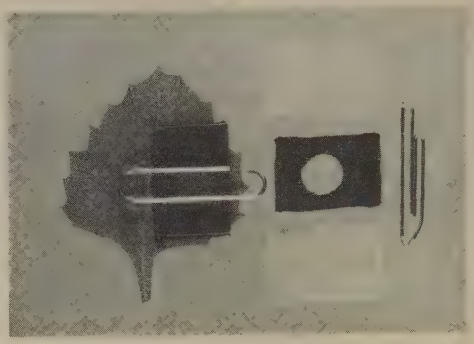


FIGURE 1. (left) Plastic cylinder cage for confining a single potted Emilia plant. Left -- to cement the top cover, a beaker is inserted. Right -- a completed cage.

FIGURE 2. (above) Felt cage for restricted feeding or serial transfers.

useful and versatile. A plastic sheeting[3] of 10-mil thickness, 9 x 10 inches, is rolled into a cylinder 9 inches high and 3 inches in diameter. For fastening, acetone is used. A window, 1.25 x 1.75 inches, is cut off near the base, and the top opening and window are covered with dacron. The top cover is readily fastened (Fig. 1) by placing a dacron piece on the bottom of an inverted 400-ml beaker which is snuggly fitted to the cage, and pushing the top opening of the cage down over the bottom of the beaker for about 1/4 inch, and dropping a few drops of acetone over the dacron piece. The excess portion of dacron extending beyond the cage margin is trimmed off and the margin is re-enforced by binding with 1/2-inch plastic tape.

To transfer thrips into the cylinder cage, they are collected into a small vial and dropped from this into the bottom opening of the cage which is held upside-down; then the cage is quickly put over the plant and pushed into the ground to a depth of 1/2 to 3/4 inch. Transfer may also be effected by placing the loaded vial on the ground near the plant. For removing thrips from the cage, the plant is first snipped off at ground level while lifting the cage slightly. The plant still encased in the cage is moved over black or white paper on which living insects are collected with an aspirator.

For restricted confinement, various modifications of the sandwich cage are useful. One the writer used for a long time is a felt cage (4, p. 888; 5, p. 10) consisting of two small pieces (3/4 x 1 inch) of plastic sheeting (15 mils or thicker), a piece (5/8 x 7/8 inch) of black felt with a central cut-out of 3/8-inch diameter, and a No. 1 size stationery clip which is reformed by pliers to clip together 1/8-inch thickness with even pressure distribution (Fig. 2). Escape of thrips may result from insufficient or uneven distribution of pressure induced by the clip. After the whole assemblage is constructed, the top plastic piece is slid partly aside to expose a part of the central cut-out of the felt piece. Then the insect is introduced into the cut-out opening with a moistened brush. As soon as the insect is released from the brush the top plastic piece is quickly slid back, and the clip is applied. For removing thrips from the cage, an aspirator is inserted into the central opening before the cage is disassembled. A wire support standing on the ground is required for hanging the cage on a potted plant.

REARING METHODS ON DETACHED PLANT PARTS

In the Berkeley project, the detached plant method was satisfactory for rearing F. occidentalis on bean pods and T. tabaci on cabbage midribs. Bryan and Smith (1, pp. 365-366) first adopted this method, but the details were not described. Erlenmeyer flasks of 125-ml

[3]Ethocel sheeting manufactured by the Dow Chemical Co. was fully satisfactory; ordinary cellulose acetate sheetings from many different sources had a poisonous effect upon plants and insects confined by such material.

size laid sidewise, and glass or lusteroid tubes, 1 x 4 inches, were used for cages. Both ends of the tubes as well as mouths of the flasks are covered with dacron pieces fastened by rubber bands; cotton plugs are unsatisfactory. After the insects are dropped into the cage, a piece of food and a piece of blotting paper are inserted, and then the opening is covered. The blotting paper absorbs any liquid oozing out from the food. When the food piece has deteriorated, a new piece is added at the side of the old one which should not be removed from the cage, because eggs are laid in it. Sometimes, but not often, bean pods may rot and cause a high mortality of thrips; this is the major shortcoming of this method. Cabbage midribs, however, do not rot readily and rearing T. tabaci on these was highly practical. A razor blade dipped in Clorox is used for cutting food pieces. Bean pods are cut through the flat portion between seeds to avoid exposing the inner cavity of the pod into which thrips tend to crawl. Too large or too many pieces of food are undesirable since they cause heavy dew condensation within the cage. A 2-inch long piece of bean pod or 1/4-cubic inch cabbage midrib per cage is satisfactory. Cut and uncut onion bulbs were tried for rearing T. tabaci in the Princeton project but were not fully satisfactory.

METHODS FOR ACQUISITION FEEDING

The feeding pattern of T. tabaci larvae on Emilia leaves (Fig. 3) indicates an increased feeding rate during the second instar stage and the presence of a 2-day non-feeding period at the end of their lives (unpublished data). During this non-feeding period the larvae appear milky white in body color, and are readily separable from the feeding larvae. In Emilia (Table 2) the highest virus concentrations are present about 2 weeks after inoculation in the inoculated as well as the next two or three leaves above (unpublished data). Intervening leaves not showing symptoms or symptomless portions of the symptom-bearing leaves, as well as floral parts, were all low in virus concentration.

For acquisition feeding, three different techniques have been tried by the writer. The first is a mass rearing of an infective colony; the other two involve restricted feeding over a specific leaf area during a specific length of time. Mass rearing is accomplished by confining female adults over infected Emilia in a cylinder cage. To concentrate the feeding of the progeny larvae on leaves with the highest virus concentration, the following synchronization is made. First, the youngest two or three leaves of a young Emilia plant 4 to 5 inches high are juice-inoculated, and all the older leaves below the inoculated ones are trimmed off. One or

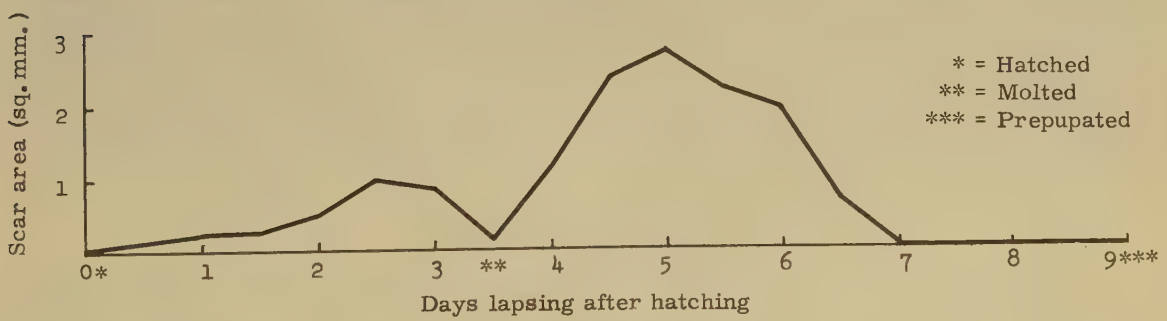


FIGURE 3. (above) Feeding scars induced in 12-hour periods by a larva of Thrips tabaci on Emilia leaves.

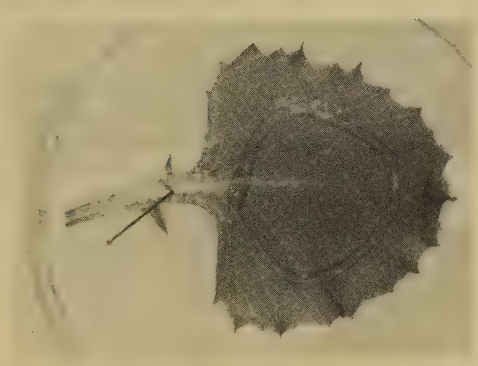


FIGURE 4. (left) Sticky ring barrier cage for acquisition feeding on a floating Emilia leaf.

Table 2. Number of local lesions on Nicotiana glutinosa induced by inoculum from Emilia with variables in leaves and days after inoculation.

Source of inoculum	Time lapsing after juice-inoculation on Emilia				
	4 days	7 days	2 weeks	4 weeks	6 weeks
Inoculated leaf[a]	2	77	216	37	2
First leaf above	-	3	159	40	-
Second leaf above	-	3	183	29	2
Third leaf above	-	-	62	18	2
Fourth leaf above	-	-	30	-	-

[a]The youngest open leaf at the time of inoculation.

2 days after inoculation two or three females are introduced into the cage. Any larger number of females may be undesirable because of too severe feeding injuries inflicted on the leaves. Under these circumstances, egg-laying is concentrated on the inoculated leaf as well as a few leaves above; thus progeny larvae hatching 4 to 5 days later are forced to feed, for the next 10 to 15 days, on the same leaves which supposedly maintain the highest virus concentration. Infective progeny adults usually appear in the cage 4 weeks after the initial transfer of the mother females. Progeny adults emerging later in the cage were found to be infective less frequently.

The felt cage method is satisfactory for restricted acquisition feeding. Another useful technique is the sticky barrier method, which confines the feeding within a sticky ring barrier on a floating leaf. Wright, et al. (12, 13) first demonstrated this technique, but only a few successful cases of acquisition were recorded. The writer used a slightly modified technique extensively for the Berkeley project (Fig. 4). The details in procedure are as follows: A thin sticky ring is made by dripping Tanglefoot, Stikem, or Stop from a match stick over a symptom-bearing area on a detached leaf. The maximum size of ring that can be made on an Emilia leaf is about 1 inch in diameter. The detached leaf is then anchored by a few pins inserted through it onto a wooden pot label fastened on the bottom of an open Petri dish, which is filled with tap water or sucrose solution to 1/8 inch above the pot label. After the leaf is stabilized on the water surface, young larvae are introduced onto the ringed area with a moistened brush. About 20 to 30 larvae per ring of 1-inch diameter should be maximum for the 2-day confinement. To reduce the incidence of larvae being trapped and killed in the sticky barrier, the whole assemblage is kept under subdued light in a cool place. At the end of the feeding period, larvae are removed with a small aspirator. Capturing should be effected by approaching the larvae from the head end by surprise. Approaching larvae at the caudal end usually ends in larvae running into the sticky barrier. With these precautions, recovery of larvae should be 50 to 90%. In the Berkeley project, when too many larvae were allowed to feed within a small ringed area, little or no recovery of infective adults was obtained. As Yarwood's experiment (14) suggests, the virus seems to be concentrated in the epidermal cells. It is quite probable, therefore, that too heavy feeding by a large number of larvae within a small limited area would result in destruction of the epidermis and consequent subminimal ingestion of virus by those larvae.

METHODS FOR TEST FEEDING

Both routine and restricted feedings are used for the test feeding. For routine feeding, a young Emilia plant with five to six leaves, individually planted and covered with a cylinder cage, is used. Insects to be tested are first examined under a microscope for identity and then are transferred into the cage. Weak vein-clearing with conspicuous twisting, the first systemic symptoms, may appear on the terminal leaf about a week after test feeding starts. To avoid too severe feeding injuries, two adults per cage are maximum for ready diagnosis of the symptoms. Before the feeding injuries become too severe, however, the test feeding should be terminated by spraying nicotine sulfate or malathion into the cage. At the end of the observation, all the plants, cages, pots, and soil should be fumigated or heated to prevent accidental contamination in the laboratory or greenhouse.

For restricted test feeding, the felt cage method is also suitable, and is fully satisfactory for the serial transfers of infective insects. Serial transfers require complicated manipulations of the test insects, and consequently loss of insects may sometimes occur. Regardless of methods used, releasing infective insects in the laboratory or greenhouse may have serious consequences. To minimize such accidents, transfers should be made during the cooler period of the day in a small compartment or separate room, which can be readily sealed off or fumigated whenever such a need arises.

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FIELD OBSERVATIONS ON THE THRIPS VECTOR SPECIES OF THE TOMATO SPOTTED WILT VIRUS IN THE SAN PABLO AREA, CALIFORNIA[1]

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Abstract

Field observations, preliminary in nature, on the ecology of the two vector species, Frankliniella occidentalis (Pergande) and Thrips tabaci Lind., of the spotted wilt virus were made in the tomato and lettuce fields in the San Pablo area. Both species are abundant from spring to fall. They are, however, differently involved in acquiring the virus from and transmitting it to a particular plant species under field conditions. The role of F. occidentalis, the western flower thrips, as the vector in California has been overemphasized, and T. tabaci, the onion thrips, probably is more important. Four new hosts in the Convolvulaceae were found.

Incidental to a related project (18) conducted at Berkeley, California for 6 months from May to November 1956, some observations were made on certain phases of the field epidemiology of the spotted wilt virus disease in the San Pablo area of the San Francisco Bay region. Data thus collected[3] are neither systematic nor complete, but they are being made available for future reference.

The disease on tomato, lettuce, and pepper has been known in California for many years, and has been studied mainly in the San Francisco Bay region. Identification of the virus, thrips transmission experiments, and other earlier observations were made by Gardner and his associates (7, 8, 9, 23) who in an abstract (7) briefly summarized their observations on spring dispersal and overwintering of the virus. Data from which their summary was drawn, however, have not yet been published. In addition to many short notes in the Plant Disease Reporter and its Supplement, incidence of the disease on tomato and lettuce in the Salinas Valley, San Pablo, Mt. Eden, and other areas in California has been reported by various authors (3, 6, 12, 16, 21). The general knowledge of the disease in California has been briefly summarized (11, 14, 22). Doutt (6) studied population development of the thrips vector in and around the tomato fields in the San Pablo and other areas, and speculated that Frankliniella occidentalis (Pergande) was the major vector species for tomatoes. These data are, however, inadequate for understanding the epidemiology of the disease in this area. Observations reported herein relate to the abundance of vector species in the field, their host preference and feeding habits, incidence of infective thrips in the field, and some weed hosts of the virus.

VECTOR SPECIES

The vector species present in California are F. occidentalis and Thrips tabaci Lind. The third species, F. moultoni Hood, mentioned in earlier references, is now recognized as one of the color forms of the F. occidentalis complex (4). The dark form corresponds to the former F. moultoni, and the pale form, the former F. occidentalis. Recent work (18) clearly demonstrates that these two color forms of the F. occidentalis complex, as well as T. tabaci, are potentially similar in virus transmitting efficiency. These two species, however, are not necessarily involved equally in acquiring the virus from and transmitting it to a particular plant species under field conditions. They have different host preferences and feeding habits, as well as different population levels with both local and seasonal fluctuations.

THRIPS POPULATIONS AND THEIR HOST PREFERENCES[4]

In late May and early June, just about the time infection begins to occur on the cultivated plants, the thrips populations were determined on 18 different weed and cultivated plant species common in and around the tomato and lettuce plantings; T. tabaci was as abundant as F. occi-

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[4]The writer was materially assisted in the familiarization of the California thrips fauna by Dr. S. F. Bailey, a resident thysanopterist.

dentalis. Mixed infestations were present on practically every plant species checked, but the differences in host preference were clearly observed. Very high populations of F. occidentalis were found on flowers of common mustard (Brassica campestris), wild radish (Raphanus sativus), alfalfa (Medicago sativa), carnation (Dianthus caryophyllus), and rose (Rosa sp.). Very high populations of T. tabaci were also present on flowers of common mustard, wild radish, dock (Rumex sp.), common vetch (Vicia sativa), and wild carrot (Daucus carota), as well as on leaves of broadbean (Vicia faba). F. occidentalis is essentially a flower feeder, but T. tabaci feeds on leaves as well as on flowers. Wild lettuce (Lactuca spp.), a common weed in the area, was only moderately infested by the predominant T. tabaci and showed only an incidental F. occidentalis on its leaves.

The population status of these thrips species on tomato and lettuce was observed from June to October. Medium to high populations were present on both plants. On tomatoes, 199 F. occidentalis and 550 T. tabaci were counted in nine different samples, the former predominating on flowers, the latter on leaves. On lettuce leaves, 11 F. occidentalis and 169 T. tabaci were counted in three samples, showing the predominance of the latter species.

Seasonal development of thrips populations in tomato and lettuce fields could not be studied by the writer. However, two references (4, 6) on this subject are available. In 1939 Doutt made observations at San Pablo and Mt. Eden, 25 miles south of San Pablo in the San Francisco Bay region. Thrips were counted on flowers and leaves of lamb's quarter (Chenopodium album), common mustard, wild radish, nasturtium (Tropaeolum majus), mallow (Malva spp.), and tomato. The predominant species in the over-all populations in and around the tomato fields was T. tabaci. F. occidentalis, however, predominated on mallow and tomato. What Doutt observed was generally consistent with the present observations. His data also showed that T. tabaci built up high populations earlier in the spring and persisted longer into the late summer and fall, whereas F. occidentalis maintained high populations only from May to August. Bryan and Smith (4) observed a similar trend of population development of F. occidentalis in the same area. No freezing weather occurs in this region, and both species are active throughout the winter months.

FEEDING HABITS OF THE VECTOR SPECIES

To observe feeding habits of F. occidentalis on tomato and lettuce, as well as on two common weed suscepts, wild morning glory (Convolvulus arvensis) and mallow (Malva nicaeensis), a series of food acceptance tests was conducted in the laboratory. On leaves, flowers, and fruits of tomato, adults of F. occidentalis fed freely for some time and occasionally laid eggs. Such eggs hatched and larvae fed freely, but apparently were unable to complete the immature stage on leaves or fruits of this host except with difficulty. Observations on flowers were incomplete, but it is very likely that the larval stage would be readily completed on them. Under field conditions, however, larvae were rather infrequent on tomato flowers. Somewhat similar responses of T. tabaci on the same host plant were observed earlier (17, p. 286). F. occidentalis fed and bred freely on the leaves of lettuce, wild morning glory, and mallow, but laid eggs rather sparingly on the first two plants. In parallel tests, T. tabaci behaved similarly but laid eggs more freely on these same host plants. These tests indicate that tomato is not a suitable breeding plant for either vector species and would be unlikely to serve as the source plant of the virus. The other three plant species, however, could potentially serve as the source of virus for both vector species.

Potentially, tomato could be infected either by T. tabaci or F. occidentalis, as they more or less similarly frequent this host plant. Our observations do not support the earlier belief that F. occidentalis is the major vector species (6). T. tabaci predominates on young tomato plants bearing no blossoms, as well as on the leafy parts of the older plants, whereas F. occidentalis occurs mainly on blossoms of the older plants. These differences might indicate which vector species is principally responsible for infection at different ages of the plant. Since lettuce is not a preferred host of F. occidentalis and does not ordinarily blossom in commercial fields, the major vector species may usually be T. tabaci.

INFECTIVE THRIPS IN THE FIELD

On limited occasions, adults of F. occidentalis and T. tabaci collected in the field were tested for their infectivity (19). Eight lots of F. occidentalis, as well as T. tabaci collected from the leaves and flowers of healthy as well as infected tomato plants, showed that none of the 108 of the former species or 235 of the latter species was infective. The incidence of infective

thrips present in these tomato fields was apparently negligible. This low incidence was, however, associated with about 50% infection on tomatoes in experimental plots. As the tomato plant is not a suitable breeding plant for either vector species, the infection on these tomato plants should have originated primarily on an out-field source or infected weed species growing in the same field. Confirming this conclusion, an earlier observation (16) is on record that the reduction of disease incidence in tomato fields in Mt. Eden was not at all proportionate to the control of thrips on the tomatoes.

Eleven F. occidentalis as well as 176 T. tabaci in four different lots collected from either healthy or infected lettuce in July and October were tested. None of the July collections was infective. However, substantial portions of T. tabaci (infection on 8 of 24 test plants), and some of F. occidentalis (infection on 1 of 5 test plants) of the October collections were infective. T. tabaci collected from weeds, Solanum sarachoides and Capsella bursa-pastoris, which may or may not have been infected, growing in and around the lettuce fields, were also infective (infection on 3 of 10 test plants). The present data suggest a substantial increase in incidence of infective T. tabaci in the lettuce fields in the fall months. As several lettuce crops are successively planted in this area, substantial T. tabaci populations are presumably carried over from one planting to another during the summer and fall. Thus, a gradual increase in and continuity of infective thrips populations may occur in the lettuce fields. These observations also provide reason to believe that these infective thrips, when they are disturbed as the lettuce is harvested, may also disseminate the virus to tomato and other plants growing in the vicinity. Thus, lettuce can be considered as one of the major sources of infection in this area, at least in the late summer and fall months. Limited winter plantings of head and Romaine lettuce are also present in this area. The winter lettuce is, however, less commonly infected than the summer and fall lettuce. Although the virus usually causes a rapidly advancing fatal infection on lettuce, on rare occasions some infected senescent plants, particularly Romaine lettuce, were observed lingering for a long time in the field. So it appears possible that lettuce could provide a favorable host succession for the continuity of the infective thrips over the winter months.

MIGRATION OF THRIPS

In California, mass migrations of F. occidentalis commonly occur when the host plants, on which high thrips populations have developed, are dried up, weeded, mowed and harvested, or plowed under (1, 2: p. 29). The most frequent and extensive migrations are the ones forced by mowing of alfalfa in the spring and summer months. Many crop plants, ornamentals, and weeds, whether or not the thrips' normal host, grown nearby or far-off to leeward of the prevailing wind, are often densely infested by swarms of thrips. Alfalfa has not been observed as a suscept of the virus. The only reference relating to alfalfa (Medicago sativa) is that Hean (13) observed "Medicago sp. (white clover)"[5] naturally infected in South Africa. Inoculation trials conducted in California as well as in Hawaii resulted in consistently negative reaction on four varieties of alfalfa for five different isolates of the virus, and all the recovery tests were negative[6]. So it can be assumed that the swarms of F. occidentalis originating on alfalfa are usually non-infective, except a few insects which may have acquired the virus prior to coming to alfalfa. This assumption is based on the fact that the adults are unable to acquire the virus. Little is known as to dispersal of infective F. occidentalis and T. tabaci in this area.

INFECTION ON WEEDS AND ORNAMENTAL PLANTS

The virus was recovered from a young re-growth of Convolvulus arvensis found in a tomato field. This confirms earlier observations made in Oregon (15, p. 13) and in Nebraska (20). In Nebraska, the source of infection in tomato plantings was surmised to be infected C. arvensis growing near the fields. The field symptoms on this weed were rather inconspicuous and showed as general chlorosis with mild mottling. This extremely common and persistent perennial weed was found to be also a preferred breeding plant of T. tabaci. For this reason, this weed was suspected of being a possible source plant of the virus in this area. Repeated

[5]She stated in a recent letter that "white clover" is a local name derived from a combination of the Afrikaans and English origins, and not related to Trifolium repens; the species of Medicago referred to still remains undetermined.

[6]In parallel tests, six different clones of T. repens were found in Hawaii to be equally susceptible to three different isolates of the virus. The local lesions were necrotic but no systemic invasion was observed.

searches in the tomato and lettuce fields and inoculation tests from suspicious specimens, however, did not produce any evidence that this weed was commonly infected.

The host range of the virus among the convolvulaceous plants has been unknown except for C. arvensis. With the co-operation of Dr. H. Earl Thomas, who supplied seeds of various species, juice inoculations were made on the following species: Convolvulus arvensis, C. randii, Dichondra carolinensis, Ipomoea batatas (var. Jersey B and Porto Rico), I. hederacea var. superba, I. purpurea, I. setosa, Quamoclit coccinea var. hederifolia, and Q. lobata. Infection was induced on I. hederacea superba which showed necrotic spot local lesions but no systemic symptoms, Q. coccinea hederifolia which showed chlorotic spot local lesions as well as systemic mottlings, D. carolinensis which showed chlorotic ring spot local lesions but no systemic symptoms and C. arvensis, which was highly sensitive to the virus and showed chlorotic ring spot local lesions and rarely zonated chlorotic systemic symptoms. Recovery tests indicated that the virus concentration is low in C. arvensis and consequently that this weed species may not serve as a good source plant of the virus.

Infected plants of mallow (Malva nicaeensis) were also occasionally observed in the field, and the virus was recovered from such plants. Field infection of Malva spp. in the San Pablo area is rather rare in the spring months but frequent in the late summer and fall months. In the Salinas Valley, Malva parviflora was recognized as a weed host of the virus present in lettuce fields (12) and infected plants of dahlia (Dahlia pinnata) and nasturtium were also observed. In regard to ornamental plants, the probable vector species should not be assumed to be only the flower-feeding F. occidentalis. T. tabaci breeds on many ornamentals under field conditions. The extreme diversity of its host range has been reported (10).

CONCLUSION AND SUMMARY

In the San Pablo area both vector species, F. occidentalis and T. tabaci, are abundant on various crop plants, ornamentals, and weeds from spring to fall, and are continuously but much less active through the winter months. Because of differences in host preferences, feeding habits, and population fluctuation, the two thrips species are differently involved in acquiring the virus from and transmitting it to a particular plant species under field conditions. Neither thrips species is able to breed readily on tomatoes. As a consequence, they may not acquire the virus from this source. However, a few transient adults of either species, which are similarly abundant on tomatoes, may be infective and could infect these plants. None of the collections of either species from infected or healthy tomatoes was proved to be infective. Both species, but particularly T. tabaci, readily breed on lettuce from which they may be able to acquire the virus; however, F. occidentalis does not prefer to feed on this host. T. tabaci, which predominates on lettuce, may be primarily responsible for infection of this plant. Infective T. tabaci were collected from lettuce and two weed species growing in and around lettuce fields; infective F. occidentalis were collected from lettuce, only once. In the San Pablo area, lettuce probably is one of the major source plants of the virus particularly during the late summer and fall and probably serves for continuity of the infective thrips over the winter months. Beside lettuce, some other major source plants of the virus probably are present among the crop, ornamental, and weed species in this area. However, little is known as to dispersal of infective thrips from such source plants. Thrips migrate when host plants are disturbed (5). Such flights of F. occidentalis are commonly induced by mowing of alfalfa, but the majority of such thrips in flight are presumably non-infective and not related to the dissemination of the virus because alfalfa is not a suscept of the virus. Wild morning glory and mallow are infected, but not so commonly as to consider them to be the major source of the virus. Artificial inoculation with the spotted wilt virus was successful on Ipomoea hederacea superba, Quamoclit coccinea hederifolia, Dichondra carolinensis, and Convolvulus arvensis. Various observations recorded in this paper provide reason to believe that the role of F. occidentalis as the vector of the virus in California has been overemphasized, and T. tabaci probably is more important than F. occidentalis.

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AN EXPLORATORY SURVEY FOR FLUORESCENT SUBSTANCES IN HYMENOMYCETES COLLECTED IN THE VICINITY OF WASHINGTON, D. C.

Curtis May[1]

During a study of the effect of cycloheximide on the growth of Endothia parasitica, in vitro suppression of pigment formation was observed. An extract from untreated cultures fluoresced in ultra-violet light. This observation stimulated interest in possible fluorescence of extracts of other cultured fungi and of sporophores of Hymenomycetes, which were appearing in abundance at the time. Although the study is still exploratory the results are presented now with the hope of stimulating further study by others.

Fresh sporophores were collected in late summer and autumn in 1960 and extracted immediately[2]. They were cut into small pieces and put into glass flasks. Sufficient ethyl alcohol was added to cover the material. The flasks were corked and extraction proceeded for 24 hours at 35° F. The extract was then filtered through Whatman No. 1 paper. Extracts were examined for fluorescence in ultra-violet of 3650A.

Fluorescence of the extracts was commonly associated with pigmented sporophores but not necessarily so. For example a fluorescent ethyl alcohol extract was obtained from Amanita verna, which is naturally white throughout.

Fresh sporophores of some species fluoresced markedly in the UV. In some species differences were observed between colors or intensity of colors in sections of context of cap and stipe, and between pellicle and gills and context of cap. For example, sections of specimens of Amanita verna displayed blue fluorescence under UV. Gills were light blue, context of stipe was darker blue, and context of cap was even darker blue. Variation in the fluorescent colors with age was noted in species of Boletus and Russula.

Spore prints of some species fluoresce on filter paper. Application of a colorless plastic spray to the prints to prevent their mutilation in storage did not change the character of the fluorescence of several species. Filter papers through which fluorescent extracts passed fluoresced before and after drying.

In ultra-violet light (3650A) fluorescence of the indicated color was observed in ethyl alcohol extracts of sporophores of the following species:

Blue: Amanita verna Fr., Clavaria sp., Clitocybe illudens Schw. (dried specimen), Laccaria lacata (Fr.) Berk. & Br., Lentinus edodes (Berk.) Singer (dried specimen), Lepiota naucina (Fr.) Kummer, Marasmius oreades Fr., Russula sp.

Green or Greenish blue: Amanita flavoconia Atk., A. muscaria Fr., A. rubescens Fr., Armillaria mellea (Fr.) Quél., Boletus badius Fr., B. bicolor Pk., Calvatia cyathiformis (Bosc) Morgan, Fomes robiniae (Murr.) Sacc. & D. Sacc., Lactarius colorascens Pk., Lepiota morgani (Pk.) Sacc., Polyporus frondosus Fr., P. gilvus (Schw.) Fr., P. robinophilus (Murr.) Lloyd, P. sanguineus Fr., Agaricus campestris Fr., Tricholoma rutilans (Fr.) Quél.

Yellow: Hygrophorus sp., Agaricus campestris Fr. (gills immediately after extraction), Scleroderma aurantium Persoon.

Further studies of possible production of fluorescent compounds by fungi in vitro on various media would be of interest. In the case of a fungus causing wood decay, such as P. gilvus, which produces extractable fluorescent material in culture, one wonders if fluorescent substances diffuse into the decaying wood and if they could be detected and identified. Pigmentation of some fungi in culture can be modified by altering the medium but one wonders if fluorescence is also modified.

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[2]I thank E. H. Hacskaylo, John Palmer, Ross Davidson, Paul Lentz, and John Stevenson for identification of the fungi listed.

STRIPE RUST RESISTANCE OF SUWON 92 AND ITS RELATIONSHIP TO SEVERAL MORPHOLOGICAL CHARACTERISTICS IN WHEAT[1]

R. E. Allan and O. A. Vogel[2]

Abstract

The reaction of Suwon 92 to stripe rust in the field at Pullman, Washington during 1960 differed from the reaction of Omar by a single, dominant factor. Stripe rust reaction was not associated with spike density, kernel color, glume pubescence and culm length.

INTRODUCTION

A severe epiphytotic of stripe rust, incited by Puccinia striiformis West., occurred throughout the wheat-growing region of the Pacific Northwest during 1960. The epiphytotic afforded an excellent opportunity for accurately determining the reaction of breeding material grown at Pullman, Washington to stripe rust. A foreign introduction grown in the Uniform Wheat Mildew Nursery of the United States Department of Agriculture, Suwon 92, CI 12666, was found to be highly resistant. Its level of resistance is considerably higher than that in commercially grown resistant varieties such as Brevor, CI 12385; Ridit, CI 6703; Requa, CI 11554; Rio, CI 10061; and Golden, CI 10063. Suwon 92 has been used for several years in the breeding program at Pullman, Washington as sources of powdery mildew resistance, early maturity, and semidwarf stature.

The inheritance of stripe rust resistance of Suwon 92 and its association with glume pubescence, kernel color, spike density, and culm length are reported in this paper.

METHODS AND MATERIALS

Three-hundred and twenty-nine F_3 lines of the cross Suwon 92 x Omar were grown in rows 8 feet long in the field; parents were alternated every 10 rows. Stripe rust reactions were recorded as resistant, segregating, or susceptible, and average culm length was determined for all rows. F_2 plants of the cross had previously been classified for glume pubescence, spike density, and kernel color. Suwon 92 has a lax spike, red kernel, and pubescent glumes. Suwon 92 plants in 36 rows were classified as resistant (pustle type 0) to stripe rust in the field; average culm length was 67 cm. Omar has a dense club-type spike, white seeds, and nonpubescent glumes. Omar plants in 36 rows ranged from 40 to 60% leaf area infected with stripe rust (pustle type 3-4) and were classified as susceptible; average culm length was 105 cm.

RESULTS AND DISCUSSION

The reaction of Suwon 92 to the stripe rust in the field in 1960 differed from the reaction of Omar by a single factor. The observed distribution of reaction of F_3 lines was 90 resistant, 155 segregating, and 84 susceptible (Table 1). Probability of a 1:2:1 hypothetical ratio was .20-.10. This factor is dominant because seven F_1 plants of the cross demonstrated resistance comparable with that of Suwon 92.

No associations were evident between stripe rust reaction and spike density, kernel color, and glume pubescence (Table 1). Reaction to the disease was also inherited independently of culm length (Table 2).

In addition to possessing a single dominant factor for stripe rust resistance, Suwon 92 was found to possess a single dominant factor for red seed color, P = .98-.95; a single recessive factor for lax spike, P = .95-.90; and a single dominant factor for pubescent glume, P = .98-.95.

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[1]Cooperative investigations of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture, and the Washington Agricultural Experiment Station.

[2]Geneticist and Agronomist, respectively, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Pullman, Washington. Scientific Paper No. 2131. Washington Agricultural Experiment Stations, Pullman, Washington.

Table 1. Reaction of F_3 rows of the wheat cross Suwon 92 x Omar to stripe rust and their classification for glume pubescence, kernel color, and spike density.

	Reaction to Puccinia striiformis				
Character expressed	Resistant	Segregating	Susceptible	Total rows	P. for chi square test for independence[a]
Nonpubescent glume	17	40	25	82	
Pubescent glume	73	115	59	247	
Total	90	155	84	329	.30-.20
Lax spike	24	41	18	83	
Dense spike	66	114	66	246	
Total	90	155	84	329	.70-.50
White kernel	24	43	15	82	
Red kernel	66	112	69	247	
Total	90	155	84	329	.30-.20

[a] Two degrees of freedom.

Table 2. Relation between resistance to stripe rust and culm length of F_3 rows of a Suwon 92 x Omar wheat cross.

Culm length (in cm)	Resistant	Segregating	Susceptible	Total rows
<61	3	8	4	15
61 - 68	8	11	6	25
69 - 76	8	18	14	40
77 - 84	16	36	10	62
85 - 92	23	30	19	72
93 - 100	18	37	21	76
>100	14	15	10	39

Chi square test for independence, 12 d.f., P = .70-.50.

These results indicate that no undesirable linkages would interfere with developing selections which combine the high level of stripe rust resistance as it occurs in Suwon 92 with such desirable characteristics as short culms, white kernels, nonpubescent glumes, and dense spikes.

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A NEW TYPE OF RESISTANCE IN CORN TO HELMINTHOSPORIUM TURCICUM

A. L. Hooker[1]

An apparently new type of resistance to Helminthosporium turcicum Pass. has been observed at Urbana, Illinois in a breeding program for resistance to this pathogen. Previously recognized sources of resistance show fewer lesions when inoculated than do susceptible strains, the character of individual lesions being similar on both resistant and susceptible plants.

Out of 25 or more sources of resistance being studied in hybrid combination and in segregating populations with artificial inoculations, two sources appeared to be unique. Source A was obtained from the variety Hastings Prolific and Source B from an unnamed popcorn variety from Peru.

In nine segregating F_2 populations involving these two sources of resistance and susceptible inbreds, plants were observed at pollination with chlorotic lesions. These plants were in marked contrast to plants having no lesions and susceptible plants with numerous elliptical necrotic lesions. A diffuse brown to black color soon developed in the chlorotic lesions, and after several weeks the center of the lesion became necrotic. A light green margin, however, persisted around the light tan center. Plants with lesions of this type could be easily differentiated from the susceptible segregates having the typical elongate grayish green to tan necrotic lesions (Figs. 1, 2). The resistant lesions also were smaller than the susceptible lesions but the chlorotic margin was the most obvious distinguishing characteristic.

The segregating populations were observed daily for several weeks during and immediately after pollination. Although necrosis eventually occurred in the chlorotic lesions, it was much delayed over that formed in susceptible lesions. Abundant sporulation was also observed in susceptible lesions, especially early in the morning when the leaves were still wet with dew. The necrotic portions of the resistant lesions at the same time, however, were smooth, suggesting that sporulation was inhibited. This observation was confirmed by placing necrotic portions of resistant lesions together with susceptible lesions in laboratory moist chambers. After 24 hours, many conidiophores with conidia had developed on susceptible lesions while only a few developed on the resistant lesions.

Plants of F_1 hybrids involving resistant Source A with five susceptible inbreds and resistant Source B with four susceptible inbreds all had chlorotic lesions similar to those observed in the F_2 populations (Figs. 3, 4). In these F_1 crosses the resistant chlorotic type of lesion was dominant to the susceptible necrotic lesion. Laboratory studies also showed sporulation on F_1 plants to be inhibited in a degree similar to that observed in the F_2 segregates.

Plants with chlorotic lesions were also observed in a synthetic variety produced by allowing several hybrids involving Source A and different susceptible inbreds to mate at random for one generation in isolation.

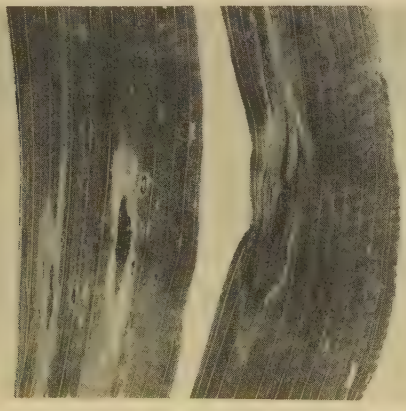

FIGURE 1. Resistant chlorotic lesions (left) and susceptible necrotic lesions (right) from resistant and susceptible corn plants in an F_2 population from the cross Source A x WR3.

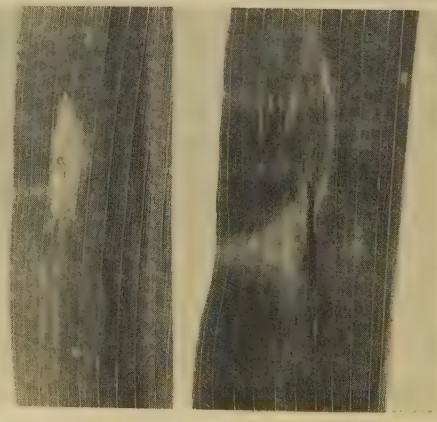

FIGURE 2. Resistant chlorotic lesions (left) and susceptible necrotic lesions (right) from resistant and susceptible corn plants in an F_2 population from the cross Source B x W187R.

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FIGURE 3. (left) Resistant chlorotic lesions on F_1 plants of the cross Source A x W22R.

FIGURE 4. (right) Resistant chlorotic lesions on F_1 plants of the cross Source B x W22R.

Table 1. Observed and expected segregations for reaction to Helminthosporium turcicum in nine F_2 populations assuming a single dominant gene for resistance (3:1 ratio) and results of the chi square tests.

Cross	Observed ratio		Expected ratio		X^2	P
	res.	susc.	res.	susc.		
Source A x WR3	132	46	133.5	44.5	0.068	.70-.80
do. x Hy-2	13	7	15	5	1.067	.30-.50
do. x W16	17	5	16.5	5.5	0.060	.80-.90
do. x W28	20	4	18	6	0.889	.30-.50
do. x M14	13	6	14.25	4.75	0.439	.50-.70
do. x Oh45	19	3	16.5	5.5	1.515	.20-.30
Source B x W64A	129	37	124.5	41.5	0.651	.30-.50
do. x W22	149	36	138.75	46.25	3.029	.05-.10
do. x W187R	141	37	133.5	44.5	1.685	.10-.20

Table 2. Observed and expected F_2 phenotypic ratios for reaction to Helminthosporium turcicum assuming a 1:2:1 genotypic ratio and results of the chi square tests.

Cross	Observed ratio			Expected ratio			X^2	P
	highly res.	res.	susc.	highly res.	res.	susc.		
Source A x WR3	36	96	46	44.5	89.0	44.5	2.226	.30-.50
Source B x W64A	35	94	37	41.5	83.0	41.5	2.964	.20-.30
do. x W22	38	111	36	46.25	92.50	46.25	7.444	.02-.05
do. x W187R	31	110	37	44.5	89.0	44.5	10.313	.01

This unique type of resistance appears to be simply inherited (Tables 1, 2). The results suggest that a single dominant gene in the heterozygous condition produces the resistant chlorotic lesion. Plants lacking this gene have susceptible necrotic lesions without chlorosis, and plants homozygous dominant are highly resistant and have few if any lesions.

Resistant Sources A and B appeared to be similar in lesion type, delay of necrosis, and inhibition of sporulation. In F_2 populations with susceptible inbreds, both sources produced the same relative frequency of plants with few if any lesions, plants with chlorotic lesions, and susceptible plants. Additional studies are in progress.

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TREATMENT OF CITRUS TREES WITH CYNEM FOR CONTROL OF RADOPHOLUS SIMILIS[1]

R. F. Suit and A. W. Feldman[2]

Abstract

O,O-diethyl O-2-pyrazinyl phosphorothioate (Cynem) used at rates of 8, 16, and 32 pounds toxicant/acre in three drench applications at monthly intervals has eradicated Radopholus similis from infected citrus seedlings under greenhouse conditions. It was more effective than 1,2-dibromo-3-chloropropane (DBCP) in stimulating renewed growth of the seedlings. Cynem at 32 pounds/acre for three applications caused some phytotoxicity. In soil column tests, three applications of Cynem at 20 pounds toxicant/acre controlled R. similis to a depth of 6 feet and at 60 pounds/acre to a depth of 9 feet which was the maximum depth that could be tested. In a field experiment, three monthly applications of Cynem at 16 pounds toxicant/acre per application gave control of R. similis for 3 months, but not after 9 months. Some increase in root and twig growth occurred on the 5-year-old citrus trees even though R. similis was not controlled.

INTRODUCTION

O,O-diethyl O-2-pyrazinyl phosphorothioate (Cynem), a relatively new nematocide, has been used to control several important plant parasitic nematodes. A soil application of a granular formulation of Cynem controlled Belonolaimus longicaudatus Rau on peanuts (1) and Meloidogyne sp. on tobacco (2). As a dip treatment for bare-root nursery stock, Cynem controlled Pratylenchus vulnus on roses but did not eliminate Meloidogyne sp. from tomato, gerbera or Shasta daisy plants (3). Also as a dip treatment, it controlled Pratylenchus sp. and Criconemoides sp. on dormant rose plants (5). Experiments with Cynem for the control of Radopholus similis (Cobb) Thorne, which causes spreading decline of citrus trees, were started at the Florida Citrus Experiment Station in 1959. 1,2-dibromo-3-chloropropane (DBCP) has shown some promise for the control of R. similis (4). In the screening tests, Cynem used as a triple treatment (three soil drenches at monthly intervals) gave results superior to those from DBCP in that a better growth response was obtained from the citrus seedlings treated with this material. This paper summarizes the current data on the effectiveness of Cynem for the control of R. similis.

MATERIALS AND METHODS

The formulation of Cynem (EN 18133) used in all experiments was an emulsifiable concentrate containing 4 pounds of toxicant per gallon and was supplied by the American Cyanamid Company.

The triple treatment (4) either by soil drench or sprinkle irrigation was the method used to apply the chemicals in the various experiments. To denote the triple treatment "3 x 8 pounds" is used which indicates three applications at monthly intervals at the rate of 8 pounds of toxicant/acre per application; or "3 x 5 gallons" which would indicate 5 gallons of toxicant/acre per application.

In greenhouse experiments, sour orange seedlings (Citrus aurantium) 4 to 6 months old were planted in containers of soil infested with R. similis in the soil temperature control tank at 75° ±2° F. Two months later groups of five seedlings were given a triple treatment with each rate of the chemicals in accordance with the procedure previously reported (4).

Soil columns were used to study the depth of penetration of the Cynem. The columns (Fig. 1) were constructed of 6-inch glazed sewer tile with T's inserted at 3, 6 and 9 feet from the top of the column. These columns were used to study the depth of penetration of Cynem in the soil. The joints were sealed and clamp coverings made for the side openings in the T's ("port holes"). The "port holes" are used for inserting or removing samples as needed and plants can be grown in them for various types of experiments. The bottom of the tile column supported on a cement block was not sealed, to permit adequate drainage. The tops of the columns were left open or covered with a plastic bag as needed for a particular experiment.

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[1]Florida Agricultural Experiment Station Journal Series, No. 1301.

[2]Plant Pathologists, University of Florida, Citrus Experiment Station.

FIGURE 1. Soil columns used for experiments on persistence and depth of penetration of chemicals in the soil. Soil samples can be obtained from 3, 6, or 9-foot depths for chemical and biological assay or plants can be grown at the various depths and subsequently examined.

The columns were filled with a Lakeland sandy soil which is typical of the so-called "Ridge District" where the largest acreage of spreading decline is found. Below 18 inches the subsoil in the field is uniform and may extend more than 15 feet before reaching the clay layer. Therefore no attempt was made to reconstruct a profile in the columns below 18 inches and they were filled with soil obtained from a depth of 3 to 6 feet. The top 18 inches of the soil profile was reconstructed in each column to resemble that found in the field.

For the field experiment, a block of 5-year-old Valencia orange trees was examined to determine those trees infected with R. similis at the start of the test and then divided into 6 plots of 32 trees each. Duplicate plots were treated with Cynem at 3 x 16 pounds and DBCP at 3 x 5 gallons while two plots were not treated. The triple treatment by the irrigation method (4) was started in May 1960 and completed in July 1960. The effect of the treatments on R. similis was determined by the examination of root samples obtained from the 12 center trees in each plot every 3 months after treatment. The effect of root growth was indicated by the variation in root profile which was determined by the dry weight of feeder roots collected from a cylinder of soil 8 inches in diameter and 6 feet deep. Root profiles were made on six trees in each plot at 3 and 9 months after treatment. The effect on top growth of the tree was obtained by measurement of 25 terminal twigs on each of six trees in each plot at selected intervals.

EXPERIMENTS AND RESULTS

Greenhouse Experiments: Two series of tests were conducted in the soil temperature control tank. The soil treatment in series A was started in May 1959, comparing Cynem at 3 x 8 and 3 x 16 pounds with DBCP at 3 x 4 gallons, and was completed in October 1959. Series B was started in August 1960, comparing Cynem at 3 x 8, 3 x 16, and 3 x 32 pounds with DBCP

Table 1. Results of tests with Cynem and DBCP for control of Radopholus similis on citrus seedlings growing at a constant soil temperature of 75° F.

Material	Rate toxicant per acre	Average per seedling: height (inches)	green weight tops (grams)	green weight roots (grams)	number R. similis
		Series A 5/19/59 to 10/9/59			
Non-treated	--	6.4	--	2.8	66
DBCP	3x4 gal.	10.1	--	3.4	0
Cynem	3x8 lb.	13.6	--	5.6	0
do.	3x16 lb.	16.1	--	11.9	0
		Series B 8/9/60 to 1/9/61			
Non-treated	--	12	20	21	64
DBCP	3x4 gal.	11	12	11	0
Cynem	3x8 lb.	26	59	40	0
do.	3x16 lb.	21	41	35	0
do.	3x32 lb.	19	37	27	0

Table 2. Studies on depth of penetration of Cynem in soil columns.

Treatment	Rate toxicant per acre	Average number R. similis per nightshade seedling roots grown in soil from depths of: 3 feet	6 feet	9 feet
None	--	105	10	25
Cynem	3x20 lb.	0	0	35
do.	3x60 lb.	0	0	0

Table 3. Results of soil treatment with Cynem on population of R. similis in infected citrus trees.

Material	Rate toxicant per acre	Average number R. similis per sample at intervals after treatment (in months): 3	6	9
Non-treated	--	13/11-58[a]	14/11-74	11/11-37
DBCP	3x5 gal.	0/11-0	0/11-0	0/11-0
Cynem	3x16 lb.	1/12-1	7/12-84	12/12-43

[a]In the fraction - Denominator = number of trees infected at start of experiment. Numerator = number of trees infected at date of sampling. Number following fraction denotes average number of R. similis in positive samples.

Table 4. Results of treatment of citrus trees with Cynem and DBCP on root and twig growth.

Material	Rate toxicant per acre	Average per 6-ft. core of soil. Gm. air dry feeder roots: 10/17/60	3/30/61	Average terminal twig growth (inches): 11/20/60	5/19/61
None - healthy	--	7.2	8.5	6.7	3.1
None - diseased	--	5.5	5.2	5.5	3.0
DBCP - diseased	3x5 gal.	5.4	6.8	4.0	2.6
Cynem - diseased	3x16 lb.	5.1	7.3	6.3	3.1

at 3 x 4 gallons. This test was completed in January 1961. Cynem applied by the triple treatment method at 3 x 8 and 3 x 16 pounds per acre controlled R. similis as effectively as DBCP (Table 1). The seedlings treated with Cynem were taller and had more roots than those treated with DBCP and the non-treated infected seedlings. In the second test, Cynem at three rates of application again controlled R. similis, as did DBCP. In this test DBCP caused stunting of the seedlings but Cynem at 3 x 8 pounds per acre doubled the growth of the seedlings. About 1 month after the third application those seedlings that received Cynem at 3 x 32 pounds per acre began to show deformation and yellowing at the leaf tips. Cynem at 3 x 32 pounds per acre also reduced the growth of the seedlings. In these containers this rate would amount to 5.3 ppm of toxicant in the soil per application.

Soil Column Experiment: After the soil in the columns had settled and had been watered for about 2 weeks, 1400 ml of infested soil and R. similis infected citrus roots was placed in the "port holes" of the columns. On March 8, 1960, two of the soil columns were treated with Cynem at the rate of 20 and 60 pounds toxicant per acre. All rates were applied in 250 ml of water and washed in with 500 ml of water. The check columns received water only. Approximately 2 inches of water was irrigated onto the soil surface of the columns each week for the duration of the experiment. Two additional applications of Cynem were made on March 29, 1960 and April 19, 1960, making a triple treatment. On April 28, 1960, citrus roots and soil of sufficient volume to fill two 6-inch pots were removed from each "port hole." These pots were planted with nightshade seedlings (Solanum nigrum) to determine the presence or absence of R. similis. The roots of these nightshade seedlings were examined for burrowing nematodes 3 months later. Cynem used in a triple treatment at the rate of 3 x 20 pounds controlled R. similis to a depth of 6 feet (Table 2). When the rate was increased to 3 x 60 pounds, R. similis was controlled to a depth of 9 feet, which was the maximum that could be tested. Considering a 10-foot soil profile the lower rate of each application was equal to 0.5 ppm while the higher rate of each application was equal to 1.5 ppm in the soil (weight basis).

Field Experiment: Root samples were obtained from the 12 center trees in each of the duplicate plots at 3, 6 and 9 months following treatment. The DBCP treated plots were free of R. similis at all sampling dates (Table 3). In the plots treated with Cynem one R. similis was found in the root sample from one tree after 3 months. However, at 6 months the effect of the chemical was no longer evident since samples from seven trees contained R. similis and 12 trees were positive at the 9-month period (Table 3).

Three months after treatment, the weights of air-dry feeder roots per 6-foot profile from the diseased non-treated and diseased treated trees were about the same but less than that of healthy trees (Table 4). This suggests that Cynem and DBCP at the rates used were not detrimental to root growth. After 9 months the amount of feeder roots per 6-foot profile had increased in the treated trees in about the same proportion as in the healthy trees. The amount of roots from trees treated with Cynem was greater than trees treated with DBCP even though the population of R. similis was increasing in the Cynem plots.

The average terminal twig growth on Cynem treated trees, 4 months after treatment, was as good as that on healthy trees and 50% greater than the twig growth on trees treated with DBCP (Table 4). Ten months after treatment the twig growth from trees in the non-treated and Cynem plots was approximately 20% greater than those from the DBCP plots (Table 4).

DISCUSSION

From the results obtained to date it appears that Cynem may control R. similis when applied to soil around diseased trees by the triple treatment if a correct rate and interval of application can be determined. Whether Cynem is capable of penetrating the entire soil profile to depths below 10 feet and whether sufficient residual toxicant can be sustained at these lower depths still remains to be ascertained.

The results from the greenhouse and soil column experiments indicate that a triple treatment in which the total amount of Cynem applied is equal to a soil concentration of 4.0 to 4.5 ppm will control R. similis.

Cynem at 1.5 ppm from three applications controlled R. similis to a depth of 6 feet in the soil columns while in the field experiment a dosage of 1.2 ppm from three applications gave control for 3 months but after 6 months the effectiveness of the chemical had disappeared. It is possible that the minimum soil concentration for control might be approximately 3 ppm based on soil profile 10 feet deep assuming equal distribution throughout and sufficient residual from each application of the toxicant. Experiments are in progress to determine an effective con-

centration and whether a longer interval of time between the applications would increase the effectiveness of Cynem under field conditions.

Soil treatment in the temperature tank with Cynem at 4 ppm has resulted in exceptional growth response by the treated citrus seedlings; however, 16 ppm was toxic. It has not been possible to determine whether the growth response was entirely the result of the control of R. similis or if there was, in addition, a growth stimulation by the chemical. Additional trials are in progress. DBCP at the rates used controlled R. similis and some response in growth by the citrus seedlings had occurred in most tests.

In the field the growth of 5-year-old Valencia trees treated with Cynem was better than of those treated with DBCP. DBCP has shown longer residual control of R. similis.

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SUN SCALD OF CORN IN WISCONSIN IN 1961[1]

Paul E. Hoppe[2]

A leaf disorder in dent and sweet corn which caused much concern to Wisconsin farmers in June 1961 was diagnosed by Earl K. Wade[3] and the writer as sun scald.

Statewide distribution of the trouble was indicated by inquiries from all sections about the cause of the unusual symptoms. Later reports showed that the condition prevailed also in Illinois, Iowa, and Minnesota. A trip on June 22 across a wide area of southern Wisconsin revealed this nonparasitic disease in every field inspected. From 10 to 25% of the plants were estimated to be affected.

FIGURE 1. Sun scald injury in corn. Note the clear-cut line of demarcation between normal and diseased areas at the lower extremities of the lesions, believed to mark the portion of the leaf out of the whorl and exposed when injury occurred.

A unique and also revealing aspect was that affected plants could be readily seen in fields when viewed from east to west, but rarely when viewed from the opposite direction. The diseased plants appeared to be distributed randomly, with no apparent difference in incidence at high or low elevations. Usually only the third or the fourth leaf was affected. Leaves with typical symptoms are shown in Figure 1. The lesions were light grey or silver and occurred on either surface, but rarely on both sides of the same leaf. The discolored areas were never directly opposite but were well separated on the leaf. Usually the lesions started about midway between the base and the tip and commonly crossed the entire width. The greyish areas extended upwards for various distances, but rarely exceeded 4 inches. In occasional plants the damage was confined to the leaf tip. A constant characteristic was a clean and almost straight-line demarcation between normally green tissue and the lower extremity of the lesions (Fig. 1).

Although many observers believed this disorder to be caused by slight cold injury, the symptoms described rule out frost as a direct cause. Low night temperatures, however, followed by heavy morning dews, are believed to have been involved. Such conditions result in water congestion in the plant cells, which predisposes highly susceptible tissues to scald when exposed to a bright morning sun. The clear-cut demarcation between healthy and diseased areas at the lower edges of the lesions (seemingly marking that portion of the susceptible leaf out of the whorl and exposed when injury occurred), together with the fact that the discoloration was confined to areas exposed to the east, seems to confirm the diagnosis of sun scald. The damage was too superficial to cause any appreciable necrosis or to affect yield.

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SEPTORIA LEAF BLOTCH, IMPORTANT DISEASE OF WHEAT IN GUATEMALA

Eugenio Schieber and Astolfo Fumagalli[1]

Septoria leaf blotch, incited by Septoria tritici Rob., has become in recent years the most important disease on wheat (Triticum vulgare) in Guatemala. The disease has been severe in all wheat regions and at altitudes ranging from 6000 to 9000 feet. These regions include Olintepeque valley in Quezaltenango, Argueta in Sololá, and Tecpán in Chimaltenango.

All commercial wheat varieties including "Lerma Rojo" now widely distributed in the country are susceptible to the disease. Attention was given to Septoria leaf blotch during 1959 and 1960 in the experimental grounds of the Labor Ovalle station of the Instituto Agropecuario Nacional located in Quezaltenango; also during the early part of 1961 in Argueta Sololá, where the new variety Nariño 59, obtained through the Rockefeller Foundation program in Mexico and Colombia, has been planted. The disease develops most severely during the rainy season, but it was also present during the dry season of 1961 on Nariño 59 under irrigation.

Symptoms on commercial and recently introduced varieties correspond to those described by Dickson[2]. Blotches appear first as light-green spots between the veins of the leaves, later spreading rapidly to form yellow to light-brown blotches (Fig. 1). Some blotches may show a speckled appearance due to the presence of pycnidia. Macroconidia predominate and are cylindrical with rounded ends, straight, hyaline and 3- to 7-septate. Inoculum persists during the dry season on plant refuse, volunteer wheat plants in the fields, and on out-of-season on plantings under irrigation.

Data obtained in recent years at the Labor Ovalle station show that only a few introduced varieties from the United States and other countries have some degree of resistance when grown under Guatemalan conditions.

FIGURE 1. Disease symptoms on leaves of Nariño 59.

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[1]Plant Pathologist and Agronomist, Instituto Agropecuario Nacional, respectively.

[2]Dickson, J. G. 1956. Diseases of Field Crops. 2nd Ed. McGraw-Hill Co. New York. pp. 248-249.

SEASONAL VARIATIONS OF NEMATODE POPULATIONS IN SOYBEAN FIELD SOIL[1]

Virginia R. Ferris and R. L. Bernard[2]

Summary

Data on the fluctuations in the numbers of plant parasitic nematodes in soils of soybean fields were obtained over a 3-year period. Populations of nematodes of the genera Paratylenchus, Helicotylenchus, and Tylenchorhynchus built up during the growing season and reached a peak near the end of this period. Populations of nematodes of the genus Pratylenchus in the soil usually reached a peak early in the season and a decline followed. There were drops in population of most nematodes during very dry periods.

Detailed quantitative data on the fluctuations in populations of plant parasitic nematodes associated with given crop plants (1, 2) are scarce. The data given here were collected during a study of nematode populations in fields of soybeans and other crop species in rotation with soybeans. Data from soybean fields sampled at intervals during three growing seasons are presented to show the variations and trends observed.

MATERIALS AND METHODS

Most of the data presented are from soybean fields at the Agronomy Farm, University of Illinois, Urbana, Illinois. The soils in these fields are of several types, but all are dark-colored prairie silt loams. In 1958 two fields (M-2N and M-3S) were sampled at monthly intervals from June to October, and the numbers of plant parasitic nematodes determined. Similarly, in 1959 the adjacent two fields (M-3N and M-4S) were sampled each month from May to October. In 1960 two fields (M-4N and M-5S) adjacent to the fields sampled in 1959 were sampled each month from June to October. In addition, in 1958 each of six blocks in an area (in field 1101) used for the testing of nematicides were sampled three times during the growing season. Seven soybean fields elsewhere in Illinois were sampled twice during the 1958 growing season, in June and in August.

The experiment with nematicides was performed on an area 16 by 144 feet on the Agronomy Farm in Urbana. The area was divided into six blocks 16 by 24 feet. Two of the blocks were treated with 3,4-dichlorotetrahydrothiophene 1,1-dioxide (PRD)[3] at the rate of 800 pounds of the formulation per acre and two with a mixture of dichloropropenes[4] at the rate of 20 gallons per acre. Two blocks were left untreated. Soil samples were taken before treating (November 1957) and again in May, July, and September 1958.

The methods for taking soil samples, for storing and processing the samples, and for extracting and counting the nematodes were detailed elsewhere (3). Each sample consisted of 20 cores taken with a sampling tube at intervals of about 50 feet. The nematodes were extracted from a 1-pint subsample by means of a uniform method of washing through screens and resuspending the residues in Baermann funnels. The plant parasitic nematodes in two aliquots from each subsample were counted at 40X magnification on the compound microscope. The counting chamber was such that the specimens in question could be examined at 100X magnification. Species determinations were made from formalin mounts and from permanent mounts of specimens in glycerin.

RESULTS

The plant parasitic species in the Urbana soybean fields were largely of four genera: Pratylenchus, Helicotylenchus, Tylenchorhynchus, and Paratylenchus. The Pratylenchus species consisted primarily of P. hexincisus and P. scribneri plus forms similar in many characteristics to these, perhaps geographical variants of them. The species differences in this genus were not discernible at the level of magnification used for counting the nematodes. Only one species of Helicotylenchus appeared to be present, a member of the erythrinae group.

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[1]Publication No. 364 of the United States Regional Soybean Laboratory, Urbana, Illinois.

[2]Consulting Plant Pathologist, 2237 Delaware Drive, West Lafayette, Indiana; and Geneticist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, respectively.

[3]Kindly furnished by the Diamond Alkali Chemical Co.

[4]Kindly furnished by the Dow Chemical Co. under the trademark "Telone."

Two species of Tylenchorhynchus were encountered. T. martini was present in the 1958 samples (M-2N and M-3S) and in field M-4N, sampled in 1960. T. acutus occurred in the two fields sampled in 1959 (M-3N and M-4S). Several species (or geographical variants) of Paratylenchus were present. No males were observed. Because of the inadequacies of the available keys, identification of these Paratylenchus species was impossible. However, specimens of these and of the other three genera have been placed on file in the Purdue University Collection, Department of Entomology, Lafayette, Indiana.

The data are presented in Figures 1 to 7 and in Table 1. In general, populations of nematodes of the genera Paratylenchus, Helicotylenchus, and Tylenchorhynchus built up during the growing season and reached a peak near the end of this period; whereas the nematodes of the genus Pratylenchus usually reached a peak soil population early in the season and after that their numbers in the soil began to decline (Fig. 5).

Figures 1 to 4 show the effect of drought periods on the soil populations of all the nematodes investigated. In 1958 there was a very dry period in August (Fig. 8). With one exception (Paratylenchus in field M-2N) the counts decreased from July to August 1958. In 1959 the dry period was during June and most of July. In most fields there were reductions in July population counts for all genera, followed in most instances by rises in numbers in the August counts.

Additional data concerning population changes during a season were obtained for the genus Paratylenchus from the experiment with nematicides. Averages of the number of Paratylenchus per pint of soil for two replicates for each treatment and for the check blocks are shown in Figure 6. In the check blocks the number of Paratylenchus increased until May and then began to decline. The exact opposite happened in the treated blocks where a steady rise occurred after May in the PRD-treated blocks and a small rise in the Telone-treated blocks.

The proportions of adult females to the pre-adult form (3, 4) in these blocks were deter-

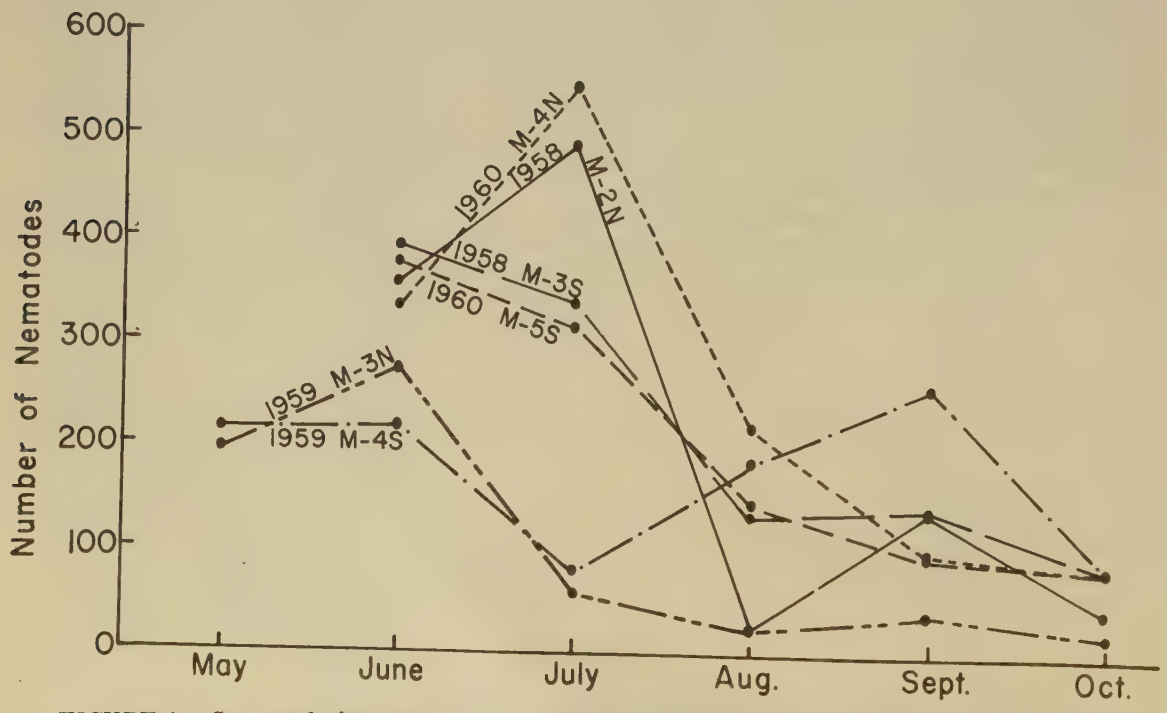


FIGURE 1. Seasonal changes of Pratylenchus populations in soybean fields at Urbana.

FIGURE 2. Seasonal changes of Paratylenchus populations in soybean fields at Urbana.

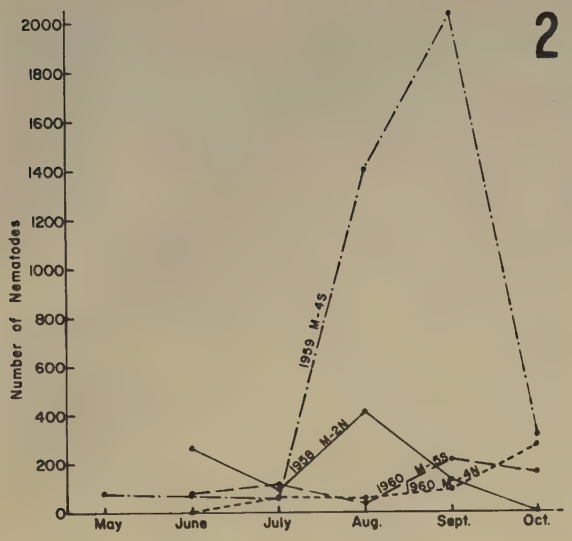
FIGURE 3. Seasonal changes of Helicotylenchus erythrinae populations in soybean fields at Urbana.

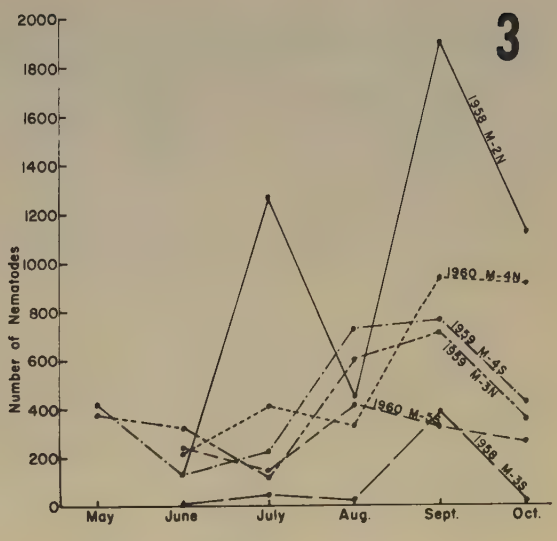
FIGURE 4. Seasonal changes of populations of Tylenchorhynchus martini and T. acutus in soybean fields at Urbana.

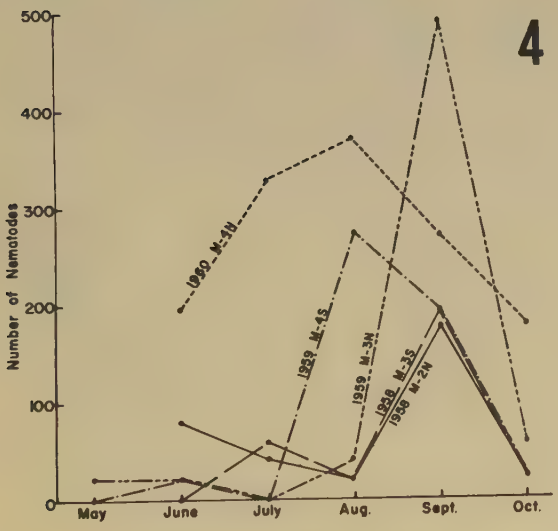
FIGURE 5. Seasonal changes of four genera of plant-parasitic nematodes as an average of six soybean fields in 3 years (1958 to 1960) at Urbana.

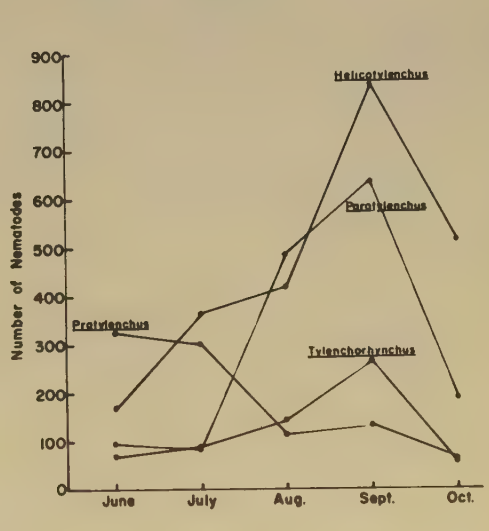
FIGURE 6. Effect of nematicides on seasonal changes of Paratylenchus populations in a soybean field at Urbana in 1957-58.

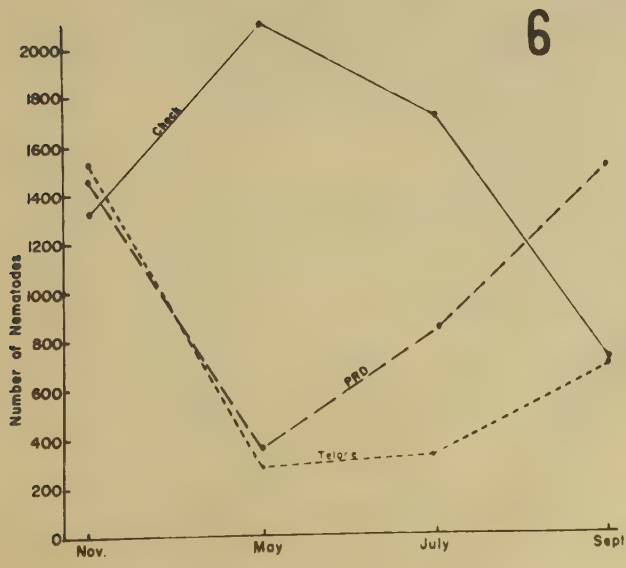
FIGURE 7. Seasonal changes of Paratylenchus in pre-adult and adult stages in untreated and in fumigated areas at Urbana in 1957-58.

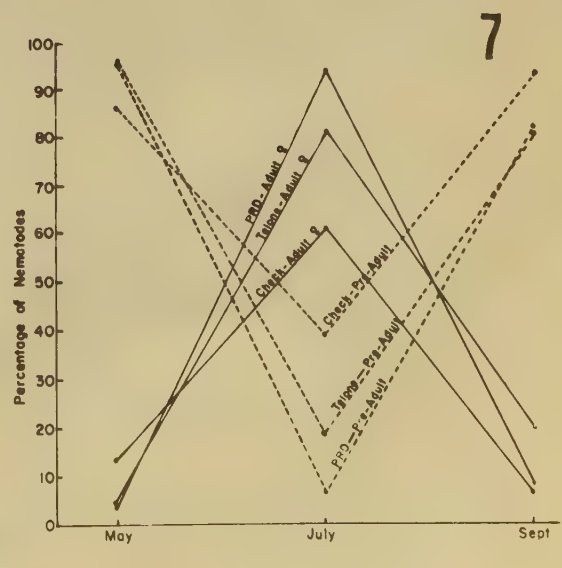












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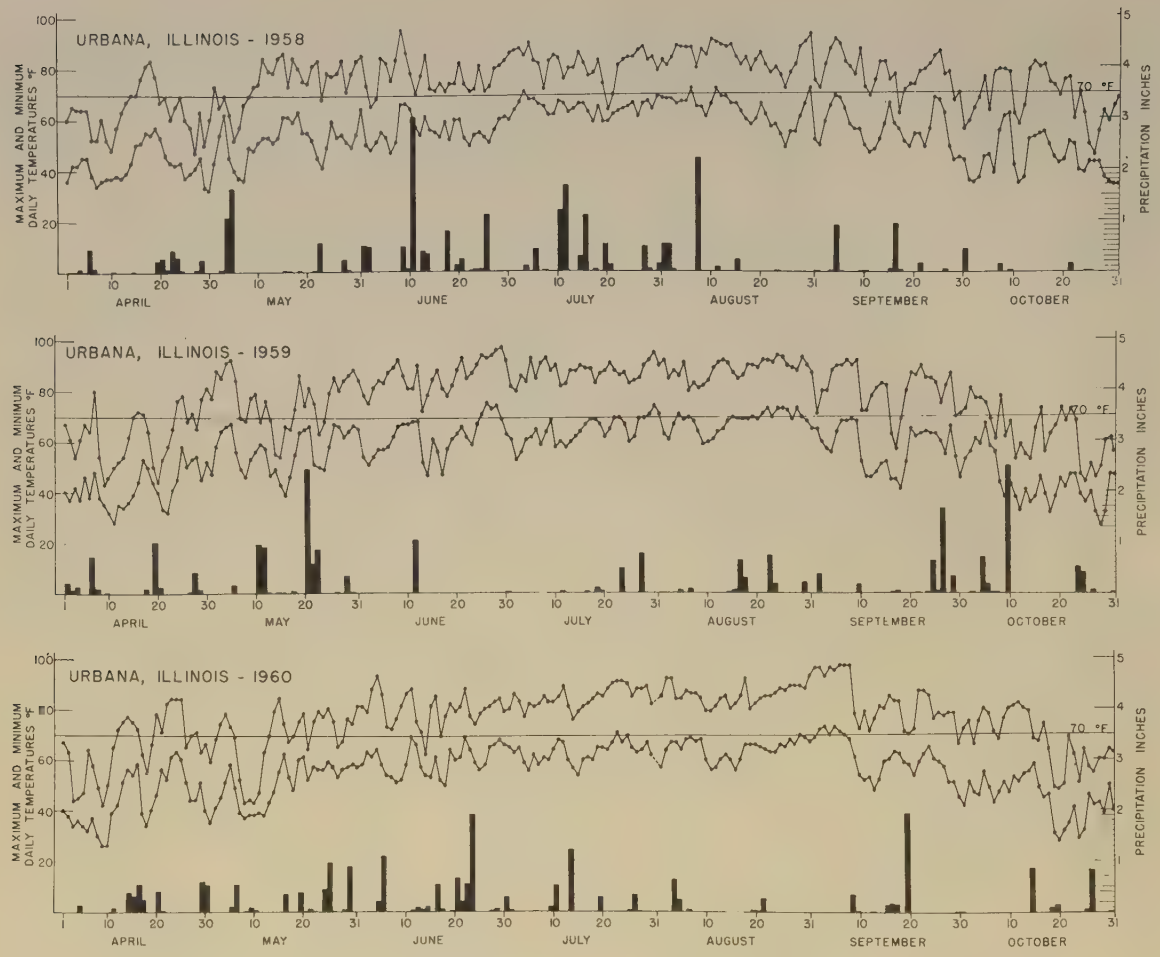

FIGURE 8. Temperature and rainfall during the growing season at Urbana, Illinois, 1958 to 1960.

Table 1. Nematodes per pint of soil, 1958 Illinois soybean test plot locations.

Location and month sampled	Pratylenchus	Helicotylenchus
Shabbona:		
June	59	373
August	40	3158
Dwight:		
June	294	118
August	20	177
Girard:		
June	177	0
August	118	216
Edgewood:		
June	157	0
August	0	0
Eldorado:		
June	304	186
August	275	1275
Ullin:		
June	196	196
August	59	883
Miller City:		
June	118	245
August	20	197

mined. Figure 7 shows that at the beginning and end of the season the pre-adult form predominated over the adult; whereas during the peak of the growing season the adult female was the predominant form.

Counts of Pratylenchus and Helicotylenchus in June and August 1958 from soybean fields throughout Illinois are given in Table 1. In most of the fields the numbers of Pratylenchus were less in August than in June and the numbers of Helicotylenchus recovered in August were greater. Root samples were taken from the Eldorado field in August at the time the soil sample was taken. From 2000 to 6000 Pratylenchus per 3 grams of roots (incubated for 1 week) were recovered from seven varieties. The average recovery was 3000 Pratylenchus per 3 grams of roots.

DISCUSSION AND CONCLUSIONS

In general the data presented confirm what might be expected with respect to soil population changes during a growing season. That is, the numbers of most of the nematodes increased during the growing period to reach a peak in early fall. The drop in population during very dry periods followed by a subsequent rise also might be anticipated.

Even the genera Helicotylenchus and Tylenchorhynchus, which were found by the authors (3) to be more prevalent in corn preceding soybeans than in soybeans, are shown here to be capable of considerable buildup in a field of soybeans. Since the fields were kept in a nearly weed-free condition it is likely that these buildups were on soybean roots.

The somewhat different development of the pattern of soil population of Pratylenchus must be explained by the fact that this is the only endoparasite in the group. The results reported here are similar to those of Di Edwardo (2), who studied the soil population changes of P. penetrans during one growing season of strawberries in New Jersey. He found that the soil population of this species of Pratylenchus reached a peak in June, followed by a decrease. A comparison of the figures showing recovery of Pratylenchus from the field near Eldorado, Illinois (Table 1) and the figures given above for recovery of nematodes from root samples collected in the same field in August are proof that though the soil count may be low in late summer, the nematodes may be present in roots in great numbers. The decrease in soil population throughout the summer undoubtedly occurs as more nematodes enter roots, and the total number may be increasing, although direct comparisons between soil and root counts may not be made.

An explanation for the Paratylenchus curves in the nematicide experiment (Fig. 6) may be that a naturally occurring agent of control was present in this field, but was destroyed in the treated area. A clue to the behavior of the Paratylenchus population during the August dry period of 1958 (Fig. 2) may be found in the nature of the pre-adult form. Rhoades and Linford (5) have shown that pre-adults are more tolerant of desiccation than other stages. Thus it might be expected that drought would not reduce the numbers of this form recovered. In field M-2N the proportion of pre-adults increased from 60% in July to 86% in August.

In conclusion, the soil populations of the four genera of nematodes appear to exhibit certain trends during a soybean-growing season. However, the population changes brought about by weather and other local conditions in most cases cannot be predicted. Such changes must be considered and understood if valid studies of population dynamics are to be carried out over a period of years. To have sufficient data to make intelligent comparisons, a researcher needs a procedure involving frequent samplings.

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SYSTEMIC CONTROL OF POWDERY MILDEW OF ROSES, CAUSED BY THE FUNGUS SPHAEROTHECA PANNOSA, WITH DERIVATIVES OF CYCLOHEXIMIDE[1]

Brian M. Jones and H. G. Swartwout[2]

Abstract

The semicarbazone, thiosemicarbazone, acetate, acetoacetate, oxime and methylhydrazone derivatives of cycloheximide were evaluated for systemic protection against powdery mildew of roses. Although soil drenches of all six materials provided protection from powdery mildew, some phytotoxicity was observed at effective concentrations of all the derivatives.

The semicarbazone, thiosemicarbazone, acetate, acetoacetate, oxime and methylhydrazone derivatives of cycloheximide[3] were evaluated for systemic protection against powdery mildew of roses. In a previous investigation[4] with the semicarbazone derivative, it was reported that this material gave complete protection from powdery mildew of roses at low concentrations with no apparent phytotoxicity. Therefore, it seemed likely that some of the other cycloheximide derivatives would show similar activity.

Soil drenches of the derivatives were applied to own-root plants of the Snow White variety of roses grown in 6-inch pots. Concentrations of solutions of the derivatives used in this investigation were 5, 10, 30, 60 and 100 ppm. Complete solubility of these derivatives was obtained by the use of a minimal amount of dimethyl sulfoxide. The resultant mixture was diluted with ethyl alcohol to give 20 ml of solution or 1% of the final volume. Enough distilled water was added to the alcoholic solution to give 2000 ml of a 100 ppm solution. This solution was either applied directly or further diluted to give the desired concentration. Two hundred milliliters of each concentration were applied to each of three plants. After sufficient time had been allowed for uptake of the derivatives, the plants were placed under rose plants heavily infected with mildew.

The six derivatives were run in two lots. Lot A consisted of the acetate, semicarbazone and methyl hydrazone derivatives. Lot B consisted of the acetoacetate, thiosemicarbazone and oxime derivatives. Nine untreated control plants were included in each lot. Three days were allowed for absorption and translocation of the derivatives included in Lot A, while 2 days were allowed for uptake of the derivatives included in Lot B. The plants treated with the derivatives in Lot A were exposed to the inoculum for 4 days, while those in Lot B were exposed for 3 days. The time interval between the start of the inoculation period and the recording of disease incidence was 9 days for Lot A and 12 days for Lot B.

When disease development was noted on untreated control plants, intensity of disease incidence was determined. For each plant, total lesions, total leaflets of susceptible age (as indicated by the position of infection on control plants), and infected leaflets were counted. Approximately 175 leaflets were examined for each three-plant treatment. Percentage control for each treatment is presented in Table 1. Percentage control was calculated by subtracting percentage infection from 100. Percentage infection was calculated by dividing the number of lesions per leaflet for the treatment by the number of lesions per leaflet for the control for the lot in which the treatment was included and multiplying this quotient by 100. Lesions per leaflet for each treatment were calculated by dividing total lesions by total leaflets examined.

As shown in Table 1, all of the derivatives exhibited a high degree of control at the higher concentra-

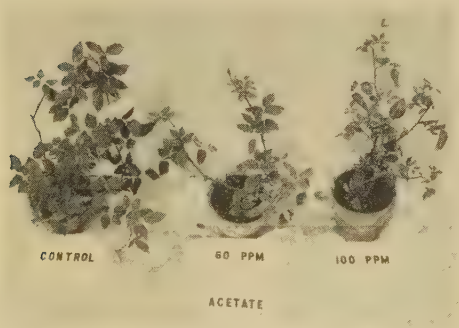

FIGURE 1. Comparison of Snow White rose plants treated with soil drenches of cycloheximide acetate at 60 and 100 ppm with an untreated control plant, showing the severe injury produced by this derivative.

[1]Contribution from the Missouri Agricultural Experiment Station, Journal Series No. 2359. Approved by Director.

[2]Department of Horticulture, College of Agriculture, University of Missouri, Columbia.

[3]Cycloheximide derivatives were furnished by the Upjohn Company of Kalamazoo, Michigan.

[4]Jones, Brian M., and H. G. Swartwout. 1961. Systemic control of powdery mildew of roses (Sphaerotheca pannosa) with semicarbazone derivative of Acti-dione. Plant Disease Reptr. 45: 366-367.

Table 1. Incidence of powdery mildew[a] and phytotoxicity on Snow White roses treated with soil drenches of derivatives of cycloheximide.

Treatment		% control[b]	Lesions per leaflet	Toxicity[c]
Lot A				
Control		0	5.82	
Semicarbazone	5 ppm	56.19	2.55	none
	10	76.29	1.38	none
	30	97.25	0.16	slight chlorosis
	60	99.93	0.004	Molderate chlorosis, lfts. stunted
	100	98.80	0.07	chlorosis, marginal necrosis, lfts. stunted
Methyl hydrazone	5 ppm	31.96	3.96	none
	10	39.52	3.52	none
	30	79.38	1.20	none
	60	71.31	1.67	none
	100	87.29	0.74	slight chlorosis
Acetate	5 ppm	64.43	2.07	none
	10	84.88	0.88	chlorosis, marginal necrosis
	30	97.77	0.13	pronounced chlorosis, lfts. stunted, marginal necrosis
	60	95.53	0.26	pronounced chlorosis, lfts. stunted, marginal necrosis
	100	99.66	0.02	pronounced chlorosis, lfts. stunted, marginal necrosis. Some intervein necrosis of older lfts.
Lot B				
Control		0	1.93	
Acetoacetate	5 ppm	52.33	0.92	moderate chlorosis
	10	91.23	0.15	moderate chlorosis lfts. stunted
	30	96.37	0.07	moderate chlorosis lfts. stunted
	60	94.30	0.11	chlorosis, lfts. stunted
	100	97.41	0.05	Severe chlorosis, lfts. stunted, marginal necrosis
Thiosemicarbazone	5 ppm	4.15	2.01	none
	10	51.30	0.94	slight chlorosis
	30	82.38	0.34	slight chlorosis
	60	97.93	0.04	moderate chlorosis
	100	96.89	0.06	moderate chlorosis, lfts. stunted
Oxime	5 ppm	27.46	1.40	none
	10	77.20	0.44	none
	30	94.82	0.10	slight chlorosis
	60	91.71	0.16	slight chlorosis
	100	98.45	0.03	moderate chlorosis

[a] Composite of three plants for each treatment, except for controls, which consisted of nine plants for each lot.

[b] % infection (lesions per leaflet for the treatment divided by lesions per leaflet for the control, and this quotient multiplied by 100) subtracted from 100. Lesions per leaflet were calculated by dividing total lesions by total leaflets examined.

[c] Description is of younger leaflets, unless otherwise designated.

tions. However, some phytotoxicity was observed at the higher concentrations of all of the derivatives. Phytotoxicity occurring at effective concentrations (arbitrarily selected as the concentration which gave approximately 90% control) of the six derivatives was compared visually. The oxime, semicarbazone, and methylhydrazone derivatives provided approximately 90% control of powdery mildew with only a slight chlorosis of the younger leaflets. The acetoacetate and thiosemicarbazone derivatives produced a moderate chlorosis at their effective concentrations, while the acetate produced severe chlorosis, stunted leaflets and marginal necrosis. Figure 1 shows the severe injury produced by the acetate derivative.

Although protection from powdery mildew was provided by the six cycloheximide derivatives tested, some phytotoxicity was observed at effective concentrations of all these materials.

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THE OCCURRENCE OF APRICOT RING POX VIRUS IN PLUM IN CALIFORNIA[1]

H. Keith Wagnon, James R. Breece, Jack A. Traylor, and Harold E. Williams[2]

Summary

Leaf symptoms believed to be caused by virus infection were found in 15 Wickson (P. salicina x P. simonii?) plum trees in 1953 and 1954 in an inspection of nursery budwood source trees in a commercial planting in Sutter County, California (13). The planting was originally of the Formosa variety, but the trees subsequently were topworked by grafting to the Wickson variety. The leaf symptoms were unlike any described for plum. Preliminary transmission tests on plum showed that a virus was present, and subsequent tests on apricot indicated that the causal agent was apricot ring pox virus.

INTRODUCTION

Apricot ring pox is a virus disease which may render the fruit crop worthless in some apricot varieties (3). When infected, the leaves of certain apricot varieties develop irregular rings, angular spots and marked clearing of the principal veins and the fruits develop reddish-purple spots and rings in the peel and tissues under the peel. The disease first was observed in Colorado in 1935 (1) and subsequently was found in Washington in 1939 (9) and in British Columbia in 1942 (6). The first known occurrence of the disease in California was a single Tilton apricot tree in 1945 in an orchard near Hemet, Riverside County (4). Further investigation of apricot orchards in the Hemet area was made in 1952, when more than 100 infected trees in four additional orchards were found. Subsequently the disease has spread rapidly in the Hemet area. During budwood source inspections carried on in 1953, a single infected apricot tree was found in a trial planting near Fresno, Fresno County (14)[3]. More recently, Nyland and Thomas (8) reported the occurrence in the Niles area, Alameda County, California of a new strain of ring pox virus. This strain, which first was noted in 1954, produced leaf and fruit symptoms on Royal (Blenheim), a variety considered a symptomless carrier of the virus as it occurs in Colorado and Washington (4, 11, 12). The three above-mentioned reports of ring pox are the only known natural occurrences of the virus in apricot in California. This paper reports the association of apricot ring pox virus with leaf symptoms occurring on Wickson plum trees in a commercial planting in Sutter County, California.

TRANSMISSION TESTS

The first evidence that the leaf symptoms on the Wickson plum were the result of virus infection was demonstrated in two Burmosa plum trees that were graft-inoculated with buds from infected Wickson trees in 1953. The two Burmosa plum trees developed similar leaf symptoms the following growing season.

Host range transmission studies of the Wickson plum virus were made with various stone fruits, including apricot. Leaf symptoms characteristic of apricot ring pox were noted on Perfection, Moorpark and Tilton apricot varieties in 1958; typical ring pox fruit and twig symptoms also were present on the Moorpark and Tilton varieties. Royal (Blenheim) variety failed to develop symptoms during transmission tests; however, this variety has been shown to be a symptomless host of the virus as it occurs in Colorado (12), California (4) and Washington (11).

Transmission of ring pox to apricot was successful from four of the Wickson plum trees in the Sutter County commercial planting. The virus also was recovered from shoots from the Formosa interstocks on three of four trees topworked to Wickson.

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[1]Information included in this paper was obtained through work done under Agricultural Marketing Service Project number SDA-Calif. -A-2, for which State funds were matched with Federal funds received from the Agricultural Marketing Service, United States Department of Agriculture, under provisions of the Agricultural Marketing Act of 1946.

[2]Respectively, Plant Pathologist, former Assistant Plant Pathologist, and Plant Pathologists, Bureau of Plant Pathology, California Department of Agriculture, Sacramento.

[3]The writers are grateful to Dr. L. C. Cochran, now Chief, Fruit and Nut Crops Research Branch, Crops Research Division, United States Department of Agriculture, for making transmission tests while Principal Plant Pathologist, United States Department of Agriculture, at Riverside, California to confirm the diagnosis on this tree which exhibited only leaf symptoms.

The following stone fruit varieties were inoculated with this virus and no leaf or fruit symptoms developed on them: Nonpareil almond (Prunus amygdalus), Black Tartarian cherry (P. avium), Pacific plum (P. subcordata), Manchu cherry (P. tomentosa), Italian prune (P. domestica), Ace, Beauty, El Dorado, Formosa, Nubiana, Red Heart and Shiro plums (P. salicina). Healthy Wickson trees developed the characteristic leaf symptoms 1 year after being graft-inoculated in late summer. Burmosa, Kelsey and Santa Rosa plums (P. salicina) developed symptoms very similar to those produced by Wickson. In each instance the symptoms always were more pronounced in the growing season following inoculation. In subsequent years they became less evident, and sometimes disappeared. Elberta and Guame peach (P. persica) and Quetta nectarine (P. persica v. nectarina) exhibited small light-green flecks on a few leaves near the points of inoculation. None of the infected plum, peach and nectarine trees developed fruit symptoms.

DISCUSSION

The serious nature of this virus in apricot and the rapid natural spread that has occurred in the Hemet area of California justifies the need for a virus indexing program to detect carriers of the virus and to eliminate the possible distribution of infected stock into other apricot-producing areas of the State.

In British Columbia, Lott and Keane (6, 7) reported that apricot ring pox has a close association with twisted leaf virus of cherry, and they suggest the probability of the two diseases being caused by a single virus. Their work demonstrated that western choke-cherry (Prunus virginiana var. demissa) can be a symptomless carrier of these two diseases. Twisted leaf virus of cherry is not known to occur in California. Two Black Tartarian trees inoculated with the Wickson plum strain of apricot ring pox failed to develop symptoms of any kind. However, Lott and Keane have shown this variety to be a poor indicator of the twisted leaf virus.

On the basis of this work, ring pox-infected Wickson nursery stock in two nurseries, one in Los Angeles County and one in Placer County, was diagnosed and the stock destroyed during 1960 (5).

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A FLOWER ROT SYMPTOM ASSOCIATED WITH STEMPHYLIUM RAY SPECK OF CHRYSANTHEMUM

Curtis R. Jackson[1]

The Stemphylium ray speck disease of Chrysanthemum morifolium described by Tammen[2] is frequently seen in Florida plantings during the spring and summer. Symptoms of ray speck, found mainly on fully expanded ray florets, consist of brown or white necrotic specks occasionally surrounded by colored halos.

Plants in a commercial planting observed in May 1961 were found to have extensive marginal necrosis of leaves immediately below the flower heads. The cause of the leaf necrosis was related to an imbalance of available nutrients in the soil. Stemphylium floridanum Han. & G. F. Weber had, in many cases, become established in this dead leaf tissue and was producing abundant conidia. Dispersal of conidia up to the developing heads was increased by daily overhead irrigation. Heads in all stages of maturation were infected and disc and ray florets rapidly became dark brown or black. Peduncles and involucral bracts were invaded after the petals had collapsed. The symptoms (Fig. 1) are similar to those of the flower blight caused by Ascochyta chrysanthemi Stev. However, the one-sided development of infected heads that is commonly associated with Ascochyta ray blight was not observed. Conidia of S. floridanum were produced abundantly on the surfaces of decayed flowers and bracts. Ray speck symptoms were also abundant on the ray florets of many heads that had not developed flower rot.

Symptoms of flower rot were reproduced by dipping buds and mature flower heads in a concentrated conidial suspension of a floret isolate of S. floridanum and incubating at 28° C and at approximately 95% R. H. for 48 hours.

FIGURE 1. Chrysanthemum flower heads, variety Yellow Iceberg, showing symptoms of flower rot and peduncle infection.

COASTAL PLAIN EXPERIMENT STATION, UNIVERSITY OF GEORGIA, TIFTON

[1] Formerly at the Gulf Coast Experiment Station, Bradenton, Florida.

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RESPONSE OF PONDEROSA AND AUSTRIAN PINE TO SOIL FUMIGANTS AND SEED TREATMENTS[1]

John L. Weihing[2], Robert Inman[3], and Glenn W. Peterson[4]

Summary

Five chemical soil treatments and two chemical seed treatments were tested for their effect on stand and vigor of ponderosa pine (Pinus ponderosa scopulorum) and Austrian pine (P. nigra austriaca) seedlings.

There was a significant difference in response to treatment between the two pine species. Seedling vigor of ponderosa pine, as evidenced by height, growth and weight, was markedly improved by two soil fumigants, Dowfume MC-2 and Vapam; height and weight of Austrian pine seedlings were not improved by soil treatment. Stand densities of Austrian pine were increased significantly by Dowfume MC-2; stand densities of ponderosa pine seedlings were not improved by soil treatment. Ponderosa pine seed pelleted with captan and thiram resulted in more dense stands; Austrian pine stand densities were not improved by fungicide pelleting.

INTRODUCTION

The Bessey Nursery of the U. S. Forest Service located at Halsey, Nebraska produces conifers for reforestation in the Rocky Mountain Region and for the Clarke-McNary tree distribution program in the Plains States. In a recent rearrangement of the nursery old eastern redcedar hedges were removed and relocated nursery beds crossed over areas where the hedges had been. Stands of eastern redcedar and ponderosa and Austrian pine seedlings were very sparse and the trees were poor in vigor wherever there had been an old hedge or wherever hedgerow soil had been dragged onto an adjacent bed. In Bessey Nursery damping-off and root rot fungi previously have caused heavy losses of pine seedlings but not of eastern redcedar seedlings. The root lesion nematode, Pratylenchus penetrans (Cobb) Sher & Allen has been isolated from roots of eastern redcedar seedlings but not from roots of ponderosa pine or Austrian pine in this nursery[5]. Thus, soil and seed treatments to control damping-off, root rot, and nematodes were used in the reported test to determine whether reduction in stand and vigor of seedlings growing in the old hedge areas could be prevented.

EXPERIMENTAL PROCEDURE

The experiment started in May 1958 and ended in October 1959. Tests involving chemical soil treatments and seed treatments were conducted simultaneously. Species used were ponderosa pine (Pinus ponderosa scopulorum) and Austrian pine (Pinus nigra austriaca)[6]. Germination tests prior to planting resulted in 54% and 55% germination of ponderosa and Austrian pine seeds, respectively. The site of the experiment was an area from which eastern redcedar hedges had been removed. Soil texture was rather coarse loamy sand and generally very permeable. Soil pH was 6.9.

Soil Treatment: A split block design was used, with the sub-treatments arranged in a Latin square. Six soil treatments, including the check, were used. Ninety-eight percent methyl bromide and 2% chloropicrin (Dowfume MC-2), sodium n-methyldithiocarbamate (Vapam), and ethylene dibromide were applied to freshly disked and smoothed ground 5 weeks prior to planting. Dowfume MC-2 was applied to the soil under plastic cover as a volatile liquid under pressure.

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[1]Published with approval of the Director as paper No. 1092, Journal Series, Nebraska Agricultural Experiment Station.

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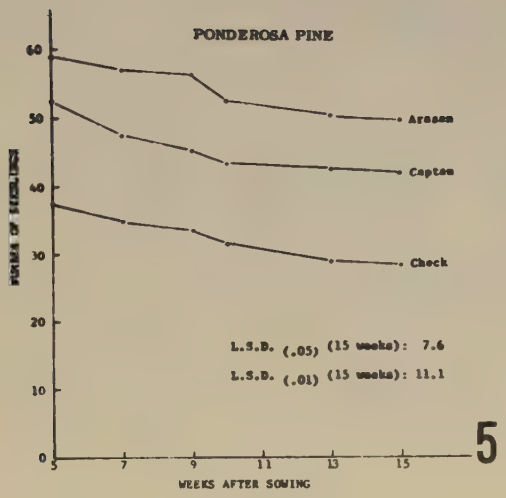
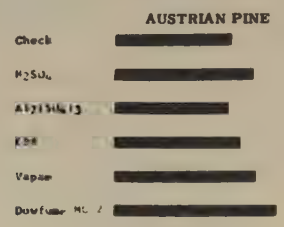
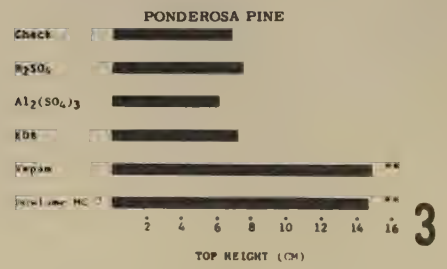
[4]Plant Pathologist, Rocky Mountain Forest and Range Experiment Station, Forest Service, United States Department of Agriculture. Central headquarters maintained in cooperation with Colorado State University at Fort Collins; research reported here was conducted in cooperation with the University of Nebraska at Lincoln.

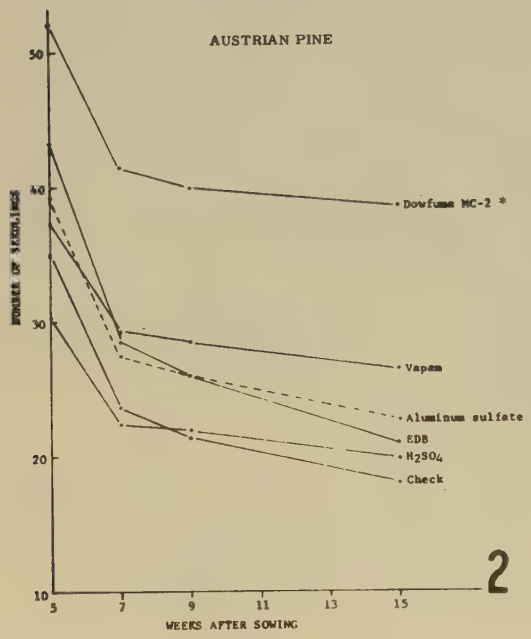
[5]Caveness, Fields E. 1957. Root-lesion nematode recovered from eastern redcedar at Halsey, Nebraska. Plant Disease Reptr. 41: 1058.

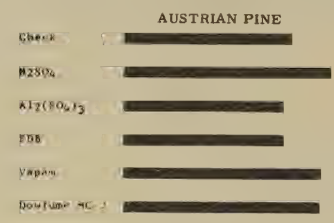
[6]Eastern redcedar was incorporated in fumigant and seed treatment tests but low germination precluded data collection.

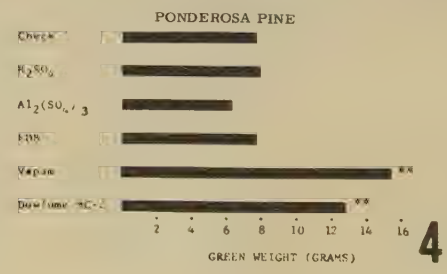


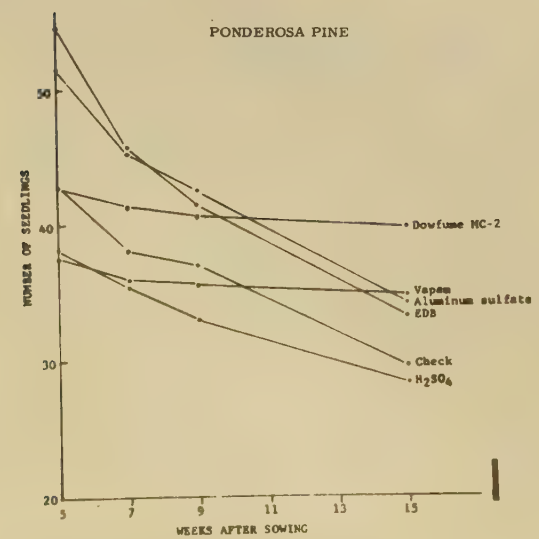


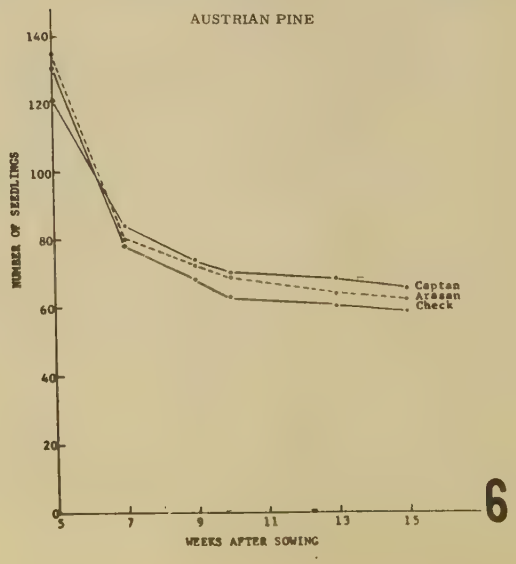












(See legends on opposite page)

The liquid was ejected from a pressurized "bomb" into a cardboard ice cream container under the plastic cover. The plastic covers were removed 30 hours after treatment. A 1-pound bomb was used on 80 square feet of soil. After being diluted with water, Vapam was applied with a sprinkling can at the rate of 1 pint/50 square feet. The Vapam-treated plots were then "sealed" by water sprinkling. Ethylene dibromide was applied at the rate of 40 ml/20 square feet to a depth of six inches by a soil injector. Twenty evenly distributed injections were delivered in each 4 x 5-foot treated plot. Aluminum sulfate and sulfuric acid were applied as soil drenches immediately after sowing. The rates according to the standard practice in use at Bessey Nursery were: sulfuric acid, 1/8 ounce/square foot in approximately 1 pint of water; aluminum sulfate, 1/4 ounce/square foot in sufficient water to dissolve it.

Seed Treatment: A split-plot design with three species (main plots), three treatments (subplots, 4 x 4 feet), and five replications was used for the seed treatment test. Seeds were coated with 50% thiram (Arasan) and 75% captan, with a 15% solution of methyl cellulose in water as a sticker. Fungicides were mixed at approximately 1:10 with the sticker in 1000-ml beakers. Seeds to be treated were immersed in the mixtures and stirred until thoroughly coated. The coated seeds were then dipped from the beakers, rolled lightly between paper towels to remove the excess fungicide, and spread to air-dry overnight. The seeds thus coated were placed in stoppered glass bottles for storage and transport. Check seeds were coated with methyl cellulose.

Planting: Seeds were handplanted singly at 3/4-inch intervals in rows spaced 3 inches apart. Because of the tedious method employed, seeding could not be completed in one operation. Approximately half of the soil treatment planting was done May 21-23, 1958. The remaining half and all of the seed treatment plots were completed June 2-4. This staggered seeding was taken into account each time seedling counts were made.

Data Collection: Stand counts were taken throughout the first summer from four 2-foot rows in each subplot. An exception was made in the Austrian pine seed treatment plots, where all of the seedlings were counted because of relatively low stand. As time passed there was some late emergence, but the late seedlings could usually be differentiated by color and texture of the hypocotyl. In such instances, late emergence was added to each previous count taken in the respective 2-foot rows. Counts were taken approximately every 2 weeks, starting 5 weeks after seeding and ending 15 weeks after seeding. Only live seedlings were counted.

FIGURE 1. Progressive stands throughout the first growing season of ponderosa pine seedlings in soils treated with fumigants and acidifying chemicals, 1958 - Bessey Nursery, Halsey, Nebraska. (Each datum is the average of the number of seedlings in four 2-foot rows in each of five replications.)

FIGURE 2. Progressive stands throughout the first growing season of Austrian pine seedlings in soils treated with fumigants and acidifying chemicals, 1958 - Bessey Nursery, Halsey, Nebraska. (Each datum is the average of the number of seedlings in four 2-foot rows in each of five replications.) *Significantly different from all other treatments, 5% level.

FIGURE 3. Top height of ponderosa and Austrian pine seedlings at the end of the second growing season, grown in soils treated with fumigants and acidifying chemicals. (Average top height of both pine species was determined from 20 seedlings from each of five replications.) **Significantly different from check, 1% level.

FIGURE 4. Green weight (tops) of ponderosa and Austrian pine seedlings at the end of the second growing season, grown in soils treated with fumigants and acidifying chemicals. (Average top weight of ponderosa pine was determined from 40 seedlings per plot, and for Austrian pine 25 seedlings per plot in each of five replications.)
** Significantly different from check, 1% level.

FIGURE 5. Progressive stands throughout the first growing season of ponderosa pine seedlings from chemically-treated and nontreated seed, 1958 - Bessey Nursery, Halsey, Nebraska. (Each datum is the average of the number of seedlings in four 2-foot rows in each of five replications.)

FIGURE 6. Progressive stands throughout the first growing season of Austrian pine seedlings from chemically-treated and non-treated seed, 1958 - Bessey Nursery, Halsey, Nebraska. (Each datum is the average of the total number of seedlings in each of five replications.)

At the conclusion of the second growing season, height and weight of a sample of the seedlings in the soil treatment plots were measured. Twenty randomly selected plants from each plot were measured for height. For weight measurements, the trees in the center 12 rows of each plot were first lifted with a regular digging blade set at a 12-inch depth. Forty randomly selected trees were taken from each ponderosa pine plot and 25 from each Austrian pine plot, the roots cut off at the ground line mark, and the green tops weighed.

RESULTS

Soil Treatment

Stand Counts: Seedling counts made in the soil treatment experiment are summarized in Figures 1 and 2. One of the replications was lost, resulting in departure from the analysis originally planned. Stand density of both species decreased in all treatments throughout the first summer from the initial density that was determined 5 weeks following planting. Although there were late emerging seedlings, they did not offset the number of seedlings that were being lost because of damping-off disease, nematodes, and other causes.

An analysis of the fifth and fifteenth week data revealed that initial and final stands of ponderosa pine seedlings were not significantly influenced by soil treatments; final stands of Austrian pine seedlings were significantly higher in Dowfume MC-2 treated plots than in plots receiving other soil treatments.

Height and Weight: Height and green top weight measurements of ponderosa and Austrian pine seedlings in the soil treatments taken at the end of the second growing season are given in Figures 3 and 4. Data analysis showed treatments significantly influenced height growth and weight of ponderosa pine, with Dowfume MC-2 and Vapam being superior; treatments did not influence height or weight of Austrian pine.

Weed Control: Weed control data were not collected; however, considerable difference was observed in weed population among the various treatments on June 25, 1958. Vapam and Dowfume MC-2 plots contained very few weeds, sulfuric acid plots a moderate number, and the other plots a dense population. All plots were hand-weeded on June 26 and regularly thereafter throughout the test period.

Seed Treatment

Treatment of ponderosa pine seed resulted in significantly greater initial and final stands (Fig. 5). There was a small, progressive loss of ponderosa pine seedlings during the summer regardless of treatment.

Fungicide pelleting of Austrian pine seed did not result in increased stands (Fig. 6). Damping-off disease continually reduced the stands of this species throughout the summer so that by the end of the growing season they were approximately one-half of the initial stands.

DISCUSSION AND CONCLUSIONS

There was a decided differential response of ponderosa and Austrian pine to chemical soil and seed treatments. Ponderosa pine responded markedly to Dowfume MC-2 and Vapam through increased plant growth; however stand densities of this species were not increased by any of the soil treatments. Austrian pine in plots receiving soil treatment did not have improved growth; however stand densities were improved by Dowfume MC-2.

The differential response between the two pine species was more marked in the seed treatment test where Austrian pine failed to show any effect of treatment, whereas stands from treated ponderosa pine seed were superior throughout the entire test.

Results indicate that soil treatment and seed treatment tests should be made for each conifer species, since the pathogenic problems of the respective species are apparently not indentical.

NEBRASKA AGRICULTURAL EXPERIMENT STATION, LINCOLN

LEPTOSPHAERIA HERPOTRICHOIDES ON RYE IN CLARK COUNTY, WASHINGTON[1]

Roderick Sprague and G. W. Bruehl[2]

A severe basal stem rot (footrot) of winter rye (Secale cereale) was found by the writers and Mary D. Glynne[3] in the plots at the Washington State University Southwestern Washington Experiment Station, Vancouver, Washington on June 26, 1959. The rye and other adjacent cereals had been given an overdose of 2,4-D weed killer earlier in the spring. Take-all (Ophiobolus graminis Sacc.) was destructive in wheat and barley, both winter and spring seeded, in the area treated. Examination of the rye plants, however, disclosed an ascomycete determined as Leptosphaeria herpotrichoides de Not. fruiting in abundance on the darkened sheaths. Lesions extended for several centimeters up the culms. In severe cases the culm tissue was partially collapsed and browned. The mycelium was not so black and carbonaceous as Ophiobolus and there was little stunting of the plant. The culms under the sheaths showed creosote brown areas and streaks. The perithecia were scattered as black, easily seen, dots over the gray sheaths.

Pure cultures were readily obtained from ascospores. The cultures grew well on PDA, producing cottony dark olive-brown colonies. These cultures were later grown for 3 to 4 weeks at a temperature approximately 50° to 60° F in flasks on sterilized sand-bran inoculum[4] and inoculated into fall seeded oats, wheat, barley and rye at the Vancouver plots. Inoculum was applied next to the base of young plants on November 4, 1959, February 16 and April 6, 1960, using the contents of one 500-cc Erlenmeyer flask to 4 feet of row.

The results of the inoculations were confused by the presence of Ophiobolus graminis in the wheat and barley and by some local Leptosphaeria in the untreated portions of the rye plots. However, Leptosphaeria spores were produced in one wheat row (Table 1) and were much more prevalent in the inoculated rye than the scattered material present in the untreated rye checks (Table 1).

While L. herpotrichoides was not nearly so severe in 1960, the fungus was moderately parasitic on rye in the area. Further study is needed on wheat and barley. However, pure culture inoculations held at 33° to 35° F in high humidity in the greenhouse resulted in only light etching of wheat plants after 3 months' incubation (February 24 to May 24).

Oats appear to be virtually immune to L. herpotrichoides and can be dropped from further consideration.

Table 1. Results of inoculations of winter cereals with pure cultures of L. herpotrichoides, Vancouver.

Host	Variety	Row number	Date inoculated	Results: injury	Results: mature perithecia
Wheat	Sun, C.I. 4509		November 4	Slight	none
	C.I. 8247	599	November 4	moderate -	+
	C.I. 5495	220	April 6	none	none
Barley	White Winter				
	C.I. 592		November 4	none	none
	C.I. 4966	282	February 16	none	none
	C.I. 9225	850	April 6	?none	none
Oats	C.I. 905	2	November 4	none	none
	C.I. 1234	5	November 4	none	none
	C.I. 708	30	April 6	none	none
Rye	Abruzzi	19	November 4	moderate	+
	Abruzzi	23	November 4	moderate	+
	Abruzzi	19	February 16	moderate +	+
	Abruzzi	23	February 16	moderate +	+
	Abruzzi	11	April 6	slight	none
	Abruzzi	25	Untreated	slight +	+

TREE FRUIT EXPERIMENT STATION, WENATCHEE, WASHINGTON
AND WASHINGTON STATE UNIVERSITY, PULLMAN

[1] Investigations by the Washington Agricultural Experiment Stations. Published as Scientific Paper No. 2153, Washington Agricultural Experiment Stations, Pullman. Project No. 796.

[2] Respectively, Pathologist, Washington State University, Tree Fruit Experiment Station, Wenatchee and Associate Pathologist, Department of Plant Pathology, Washington State University, Pullman.

[3] The help given by Miss Glynne of the Rothamsted Experimental Station is appreciated. She was unable to do further work with the organism and graciously declined a proposal of joint authorship.

[4] Equal volumes of wheat bran and river sand were mixed and moistened, then autoclaved intermittently on three successive days.

BLACK ROOT-ROT DEVELOPMENT ON PINTO BEANS, INCITED BY SELECTED THIELAVIOPSIS BASICOLA ISOLATES, AS INFLUENCED BY DIFFERENT SOIL TEMPERATURES[1]

C. R. Maier[2]

Summary

Selected isolates of Thielaviopsis basicola obtained from pinto beans were studied to determine the effect of soil temperature on the rate of black root-rot development and on the pathogenicity of the isolates. Constant soil temperature tanks set at 60°, 66°, 74°, and 80° F were employed, and a fluctuating temperature check was included with each isolate. The six isolates evaluated ranged from non- to highly pathogenic. The more virulent isolates were most destructive at 60° and 66°; their pathogenicity decreased as soil temperature increased. The relative pathogenicity of all isolates remained at distinct levels, regardless of temperature, but greater differences between severity at lower and higher temperatures occurred with the more virulent isolates. At lower temperatures, root-rot developed more rapidly from 2 to 3 weeks after emergence; at the higher temperatures it developed more rapidly from 3 to 4 weeks after emergence. Black root-rot was generally more severe at the end of the experiment than earlier.

The presence of Thielaviopsis basicola (Berk. & Br.) Ferr. in the upper Rio Grande Valley (2) and its complicity in the cotton seedling disease complex (4) makes it an economic hazard in the production of both cotton and field beans in southern New Mexico. Wiedman (5) established that the black root-rot fungus in the field bean root-rot complex contributes substantially to the losses incurred. This finding led to the present investigation of the variation among isolates within the species T. basicola. The range of isolate variation in culture and pathogenicity approximates that determined for isolates obtained from cotton seedlings reported earlier (3). The effect of different soil temperatures on cotton seedling disease symptoms produced by T. basicola was studied by Blank, Leyendecker, and Nakayama (1), but they did not consider isolate or "strain" variations.

METHODS AND MATERIALS

From investigations of the cotton seedling disease and pinto bean root-rot complexes during 1959 and 1960, 113 isolates of T. basicola were obtained and cultured. Of the 40 isolates recovered from pinto beans, 11 were selected for evaluation of pathogenic and cultural variability. Of those 11, five were evaluated for the influence of different constant soil temperatures on the rate of root-rot development and on disease severity. These isolates were as follows: 27D, highly pathogenic; 49C and 22D, moderately pathogenic; 11C, slightly pathogenic; and 1C, non-pathogenic, based on earlier evaluations.

The constant soil temperature tanks were pre-set at 60°, 66°, 74°, and 80° F. Each tank held 24 cans, four replications of the six isolates. A fluctuating temperature check for each isolate, with a temperature range from 47° to 94°, was maintained on an adjacent greenhouse bench. The entire experiment was repeated three times over a 6-month period. There was good agreement among the results of the three trials, and the data presented are those of the first trial. The experimental design was a split plot, with six isolates, at five temperatures, with four replications, and was analyzed as such at each sample period.

Sterilized bean-field soil was seeded with a culture suspension of ten commonly occurring non-pathogenic soil fungi to establish a "normal" microflora. Each can was then inoculated with 20 ml of a chlamydospore suspension of T. basicola isolates adjusted to 5×10^4 spores per ml. The non-inoculated control cans were treated with 20 ml of distilled water. After 5 days' incubation, 25 pinto bean seeds were planted per can. Virtually complete emergence had occurred by 9 days after planting, at which time the first of five weekly samples of five plants

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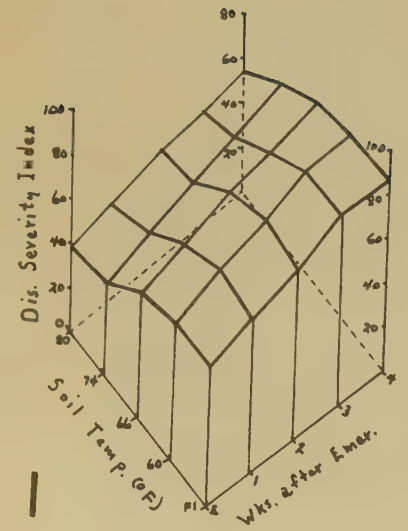
[2]Assistant Plant Pathologist, New Mexico Agricultural Experiment Station, University Park, New Mexico.



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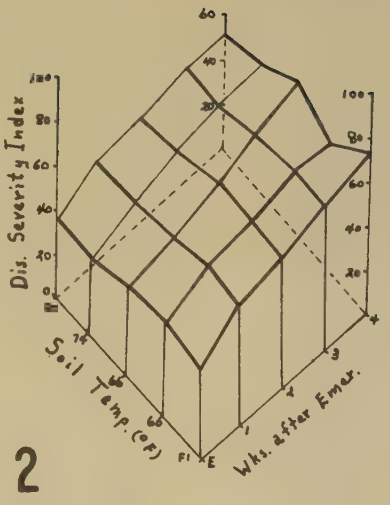

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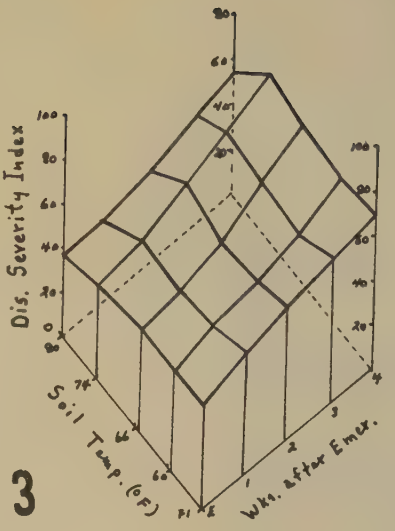

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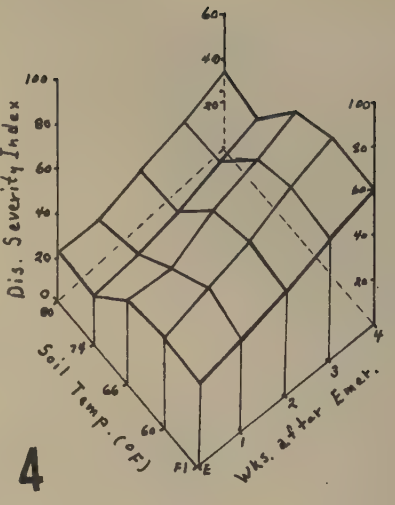

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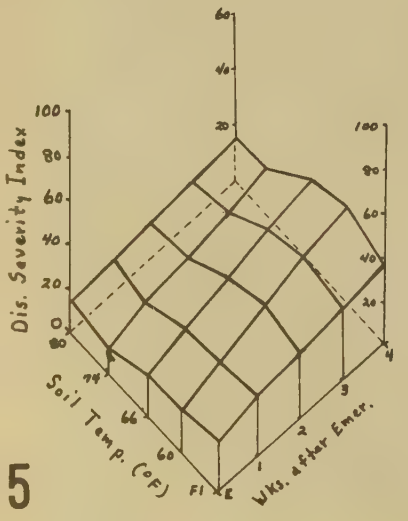

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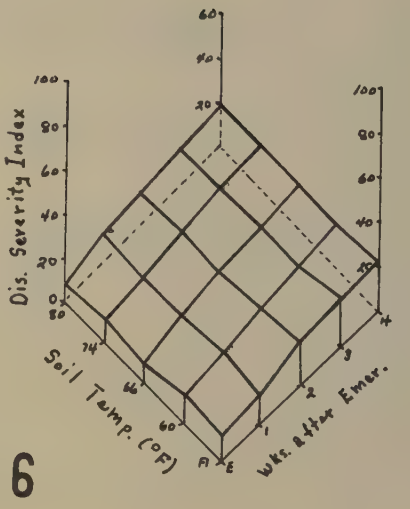
FIGURES 1 - 6. Patterns of pathogenicity for selected Thielaviopsis basicola isolates on pinto beans at different soil temperatures over a 4-week period. 1 -- Highly pathogenic isolate 27D. 2 -- Moderately pathogenic isolate 49C. 3 -- Moderately pathogenic isolate 22D. 4 -- Slightly pathogenic isolate 11C. 5 -- Non-pathogenic isolate 1C. 6 -- Non-inoculated control.



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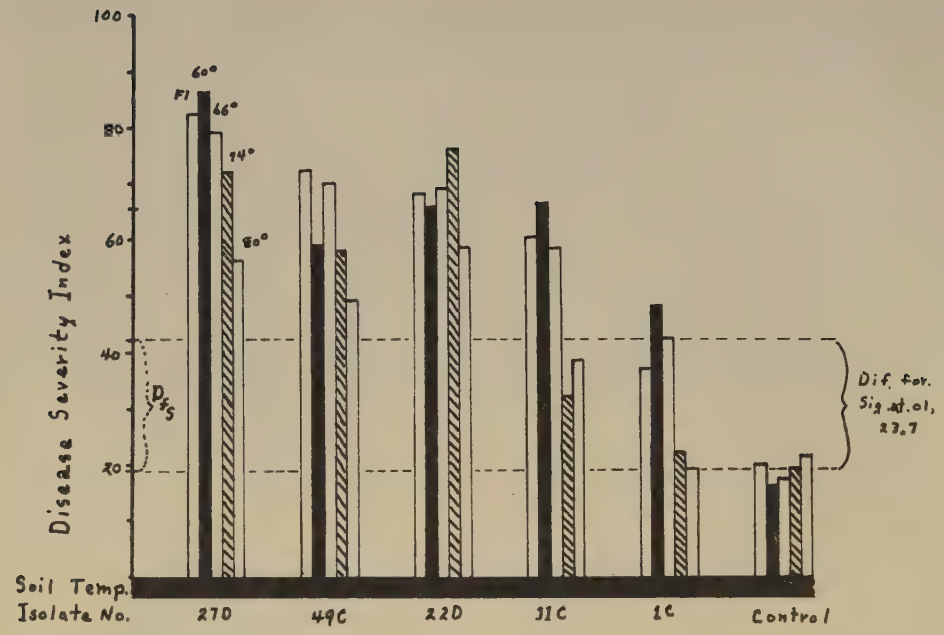

FIGURE 7. Disease severity indices for the five Thielaviopsis basicola isolates and non-inoculated control at each soil temperature at 4 weeks after the emergence of pinto beans.

each was removed from each can. The plants were visually rated for percentage infection and root-rot severity, and plant survival was recorded. Microscopic examination supplemented by tissue isolation was employed to establish T. basicola as the causal organism. Isolation from infected tissue was on potato-carrot-dextrose agar plates. A disease severity index for each sample, calculated from root-rot severity, percentage infection, and plant survival was used to express the amount of root-rot damage.

EXPERIMENTAL RESULTS

The rapidity of disease development in the fluctuating temperature checks agreed well with earlier studies for all isolates. Generally, the isolates maintained distinct levels of pathogenicity at all temperatures over the experimental period. One notable exception was non-pathogenic isolate 1C, which became slightly pathogenic at 60° and 66° F at 4 weeks after emergence. Highly pathogenic isolate 27D produced a pattern of pathogenicity at all temperatures over the experimental period (Fig. 1) which was moderate at emergence, increasing to severe at 4 weeks after emergence. Development of disease in cans inoculated with moderate pathogens 49C (Fig. 2) and 22D (Fig. 3) showed mild severity at emergence, increasing progressively to moderate severity by the end of the experiment. Slightly pathogenic isolate 11C (Fig. 4) had incited mild root-rot at emergence, which did not increase significantly in severity up to 4 weeks after emergence. Non-pathogenic isolate 1C (Fig. 5) produced mild root-rot at 60° and 66° at 4 weeks after emergence, but generally induced disease severity approximating the pattern exhibited by the non-inoculated control (Fig. 6). The overall root-rot severity pattern induced by 1C was consistently higher than the control, but not significantly so. The disease present in the non-inoculated controls was presumably due to incomplete sterilization and/or reinvasion of the soil by pathogens other than T. basicola, chiefly Rhizoctonia solani.

A pattern of more severe disease occurred at the lower temperatures (60° and 66° F) and in the fluctuating temperature check than at the higher temperature (74° and 80°). Greater differences in suppression of root-rot at the 80° temperature occurred with the more virulent isolates 27D, 49C, and 22D than with the less virulent isolate 11C. A lower severity index

pattern produced by 11C resulted at 74° than at 80°, a condition for which there appeared no plausible explanation. Isolates 27D, 11C, and 1C produced the highest severity indices over the experimental period at 60° F; a soil temperature of 66° induced the most severe disease with isolate 49C, and 74° with isolate 22D. The T. basicola damage expressed as disease severity indices for all the isolates over the temperature range employed at the end of the experimental period are presented in Figure 7. The differential response of the isolates to temperature is apparent.

Disease had developed in all cans inoculated with isolates 27D, 49C, 22D, and 11C by emergence of the beans. Considerable infection of radicles and feeder roots was in evidence at that time, as determined by microscopic examination. Generally, the disease severity increased over the experimental period, and was most severe at the end of the experiment, except for isolate 49C, which produced its greatest severity at 60° F at 3 weeks after emergence. The most rapid development of disease, as expressed by increase in severity index, occurred earlier (from 2 to 3 weeks after emergence) at the lower temperatures (60° and 66°) than at the higher temperatures. Similarly, disease induced by the more virulent isolates 27D, 49C, and 22D developed most rapidly from 2 to 3 weeks after emergence, compared with that induced by the less virulent isolate 11C, which developed most rapidly after 3 weeks. The development of disease produced by all isolates as well as its ultimate severity in the fluctuating check was closer to that at 66° than at any other constant soil temperature.

For all the isolates at the temperatures employed, there were no significant differences in the percentage of surviving plants infected, excluding the non-inoculated control. Percentage infection ranged from 75 to 89, as determined by tissue isolation. The Duncan MR test was employed to measure isolate variation at the different temperatures for each sample period, with the following differences for significance: at emergence, 17.9; after 1 week, 21.2; after 2 weeks, 19.6; after 3 weeks, 20.8; and after 4 weeks, 23.7.

DISCUSSION

Because most of the isolates of T. basicola produce more severe root-rot at lower soil temperatures, which are unfavorable for good growth of the pinto bean, the black root-rot fungus may render beans more subject to later root-rot caused by the more virulent pathogenic fungi Fusarium solani f. phaseoli and R. solani. Root-rot incited by T. basicola is most prevalent in the early part of the season, and its effect, from which most affected plants recover, is stunting and delayed plant maturity. This effect increases losses due to downgrading because of poorer quality of the harvested crop, brought about in part by exposing the maturing crop longer to the hazard of weather damage. Delayed planting, where possible, until higher soil temperatures have been attained would restrict losses due to T. basicola by favoring bean plant growth while inhibiting the pathogenicity of various strains of the black root-rot fungus.

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NEW MEXICO AGRICULTURAL EXPERIMENT STATION, UNIVERSITY PARK, NEW MEXICO

SELECTIVE EFFECTS OF BARLEY RESIDUE ON FUNGI OF THE PINTO BEAN ROOT-ROT COMPLEX[1]

C. R. Maier[2]

Summary

Barley straw incorporated into soil infested with fungi of the bean root-rot complex resulted in a selective effect on the individual fungi. When a mixed inoculum induced the disease in naturally-infested field soil, barley straw generally suppressed root-rot without any apparent effect on pathogen populations. When Fusarium solani f. phaseoli was the incitant, disease severity was sharply reduced, but soil populations of the pathogen were not affected. In barley-amended soil inoculated with Rhizoctonia solani, disease severity was markedly suppressed and soil population of the fungus was reduced. The infestation of barley straw-amended soil with Thielaviopsis basicola produced no effect on bean root-rot severity, but slightly increased soil population of the black root-rot fungus. Sharp increases in soil populations of a Chaetomium sp. (antagonistic to F. solani f. phaseoli) and three other common soil fungi occurred. Seeding of barley straw with a culture suspension of the Chaetomium sp. resulted in greater suppression of root-rot severity than did barley straw alone in sterilized soil reinfested with F. solani f. phaseoli.

The pinto bean root-rot problem in the inter-mountain areas of New Mexico has grown steadily worse over the past decade, and according to growers has played a substantial part in the diminishing bean acreage. Perennial cropping of pinto beans (Phaseolus vulgaris) has resulted in the buildup of root-rot pathogens Fusarium solani f. phaseoli (Burk.) Snyd. & Hans., Rhizoctonia solani Kuehn, and Thielaviopsis basicola (Berk, & Br.) Ferr., and led to the abandonment of the crop on many soils because of serious yield reduction.

In New Mexico, yields of field beans have been increased when the crop follows barley planted as an interim rotation crop[3]. This effect has also been reported from other areas of the western region in which bean root-rot is a problem (1,4). Greenhouse investigations have shown barley residue to be among the most effective of crop residues tested in suppressing bean root-rot (2). The influence of barley straw and other mature residues in reducing root-rot severity has been interpreted by Burke (1) and others (2, 4) as a function of the carbon to nitrogen ratio of the residues. This interpretation was supported by reports from California (6) that the suppressing effects of barley straw could be neutralized or reversed by the addition of nitrogen sources. Tolmsoff and Young (5) reached a similar conclusion in evaluating the effects of barley and oat straw on Verticillium wilt of potatoes. They pointed out that sufficient nitrogen was necessary for vigorous plant growth, however.

Certain selective effects of crops and crop residues on fungi of the soil mycoflora were obtained in other studies. Some fungi were affected in the same manner by both growing crops and their residues, while others were affected differently (3, 7). Most crops employed affected both the quantitative and qualitative composition of the soil microflora. A study was undertaken at this station to determine the interrelationships underlying the observed effects of barley residues on the severity of bean root-rot and on bean root-rot pathogens, with the hope of arriving at a plausible explanation.

METHODS AND MATERIALS

Experiments were conducted in the greenhouse to evaluate certain factors in the influence of barley residues on bean root-rot. Sandy loam soil for these experiments was collected from a heavily infested field in the Lewis Flat area near Deming, New Mexico. Evaluations were made in both naturally infested soil and reinfested, methyl bromide sterilized soil. Residues were ground to particles up to 1 mm in size in a small Wiley mill. Soil was placed in small flats, 4 x 22 x 4 inches, each holding 10 pounds, and amended at the rate of 1% dry weight of soil. Certain check flats were unamended. An artificial microflora composed of the 10 most

[1]Journal Series 170, published with the approval of the Director, Agricultural Experiment Station, New Mexico State University, University Park, New Mexico.

[2]Assistant Plant Pathologist, Agricultural Experiment Station, New Mexico State University.

[3]Personal interview with Mr. W. L. Franklin, bean grower in Lewis Flat area, near Deming, New Mexico. May 1959.

commonly occurring non-pathogens was added to the sterilized soil to reestablish a normal microflora. Pinto beans were planted 50 seeds per flat after 4 to 5 days' incubation, and four replications were employed in all phases of the investigation.

The soil fungi employed were isolated from bean field soil or bean tissues, and were maintained in culture on potato-carrot-dextrose agar. R. solani, F. solani f. phaseoli, or T. basicola inoculum for reinfestation of sterilized soil consisted of 160 ml of a culture suspension from one mature plate colony divided equally among four replicates.

Isolations to determine the effects of amendments on soil populations of fungi were made from bean rhizosphere on both Czapek's and PCDA for comparison. A modified soil dilution method was used, in which the first dilution (1:100) was made in pasteurized sand, and subsequent dilutions in distilled water. The final dilution plated was $1:10^4$. Isolations from root and hypocotyl lesions were made on PCDA plates to determine the causal organism in specific instances. Lesions were selected for isolation under a dissecting microscope. Root-rot severity ratings on a visual rating scale of 0 to 4 were made 4 to 5 weeks after planting beans. In the scale, 0 means no infection; 1, slight discoloration with scattered lesions; 2, slight rotting with moderate discoloration; 3, moderate rotting; and 4, severe rotting to death of plants. Random plants were removed at various times before the final rating to determine stages of disease development.

EXPERIMENTAL RESULTS

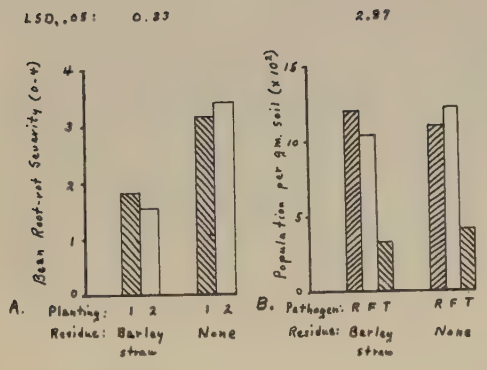
Soil dilution plate isolations from naturally infested bean field soil amended with barley straw established the presence of F. solani f. phaseoli, R. solani, and T. basicola. Root-rot severity incited by the pathogen complex was significantly less in the barley amended soil than in the unamended soil (Fig. 1A). A second planting of beans raised in the same soils gave essentially an equal degree of root-rot suppression obtained in the first planting by the barley amendment. Isolations made after the first planting demonstrated populations of the pathogenic fungi to be not significantly different in the barley amended soil from those of the unamended soil (Fig. 1B).

In a comparison of green barley residue with unamended soil in suppression of root-rot due to the pathogens individually, green barley residue did not decrease the severity of Fusarium root-rot, but did suppress Rhizoctonia root-rot in sterilized soil (Fig. 2). No suppression of root-rot severity was obtained when natural field soil amended with green barley and infested with each of the pathogens was used. Barley straw amendment suppressed the severity of disease due to Rhizoctonia and Fusarium as compared with that in unamended soil, in both naturally infested and sterilized, reinfested soils (Fig. 3). Neither barley straw nor green barley residues had any effect on Thielaviopsis root-rot in either soil. In earlier tests, green barley had given a slight increase in black root-rot severity (2).

Rhizosphere soil isolations from the foregoing trials gave the following results: barley straw amendment had no significant effect on Fusarium populations; Rhizoctonia populations were reduced; and Thielaviopsis populations were slightly increased (Fig. 4). There was no direct relationship between the effect of barley amendment in suppression of root-rot severity and on reduction of pathogen soil populations.

Isolations were made from both barley straw amended and unamended field soil where beans had developed moderate infection. Populations of Cephalosporium, Alternaria, Cladosporium, and Chaetomium were increased in barley-amended soil, while populations of Penicillium, Stachybotrys, and Gliocladium were decreased by this residue (Fig. 5). Aspergillus and Spondylocladium, two other more common fungi, were not affected in soil population by the barley amendment. These effects on soil populations of the pathogens and associated fungi, coupled with the suppression of root-rot severity except when induced by Thielaviopsis alone, suggest a selective effect by barley straw residue on the pathogens.

Isolates of the non-pathogenic fungi showing increased populations were tested in culture for antagonism to Rhizoctonia, Fusarium, and Thielaviopsis. Of over 300 tested, 45 isolates inhibited one or more of the pathogens. One Chaetomium isolate which strongly inhibited Fusarium was selected to determine the effects of seeding barley straw residue on bean root-rot suppression. Significantly greater suppression of root-rot severity occurred when the barley straw was seeded with a culture suspension of a plate culture of Chaetomium than when barley straw alone was incorporated into sterilized soil reinfested with F. solani f. phaseoli (Fig. 6A). Stronger suppression under these conditions occurred during a second planting of beans in the same soils than during the first. No significant differences in Fusarium populations was detected upon isolation from the variously amended soils (Fig. 6B).


LSD,.05: 0.83
2.87
Bean Root-rot Severity (0-4)
Population per gm. soil (x10²)
A. Planting: 1 2 1 2
Residue: Barley straw None
B. Pathogen: R F T R F T
Residue: Barley straw None

R- Rhizoctonia solani
F- Fusarium solani f. phaseoli
T- Thielaviopsis basicola

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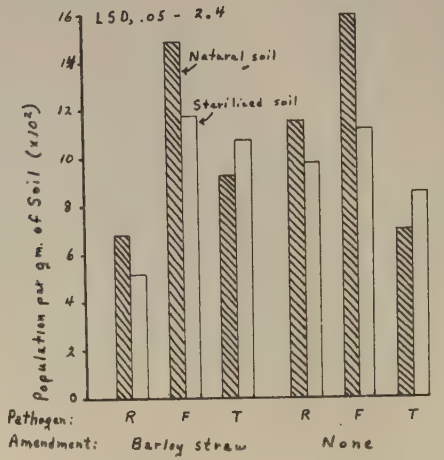

R- Rhizoctonia solani
F- Fusarium solani f. phaseoli
T- Thielaviopsis basicola

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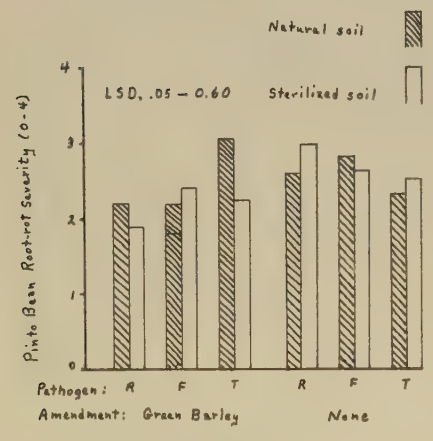

R- Rhizoctonia solani
F- Fusarium solani f. phaseoli
T- Thielaviopsis basicola

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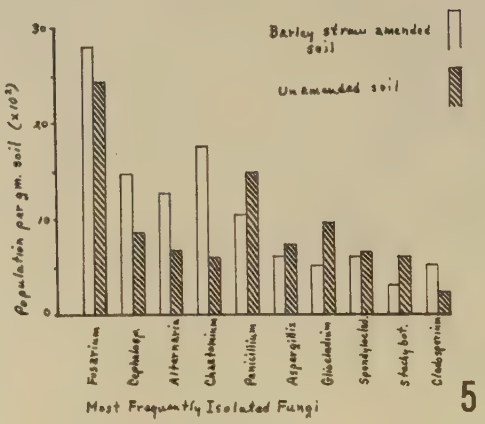

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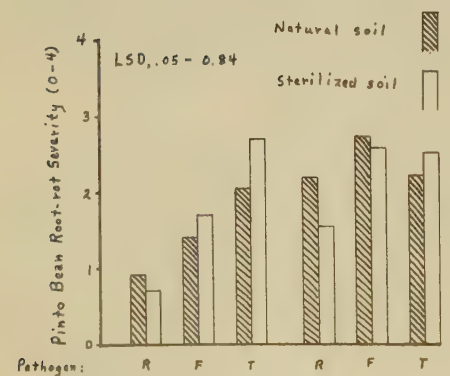

R- Rhizoctonia solani
F- Fusarium solani f. phaseoli
T- Thielaviopsis basicola

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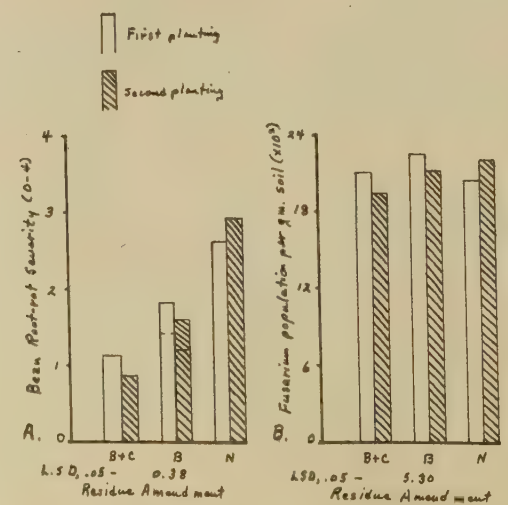

B+C - Barley straw seeded with Chaetomium sp.
B - Barley straw alone
N - No residue amendment

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(See legends on opposite page)

CONCLUSIONS AND DISCUSSION

The results indicate a selective effect by barley straw on certain components of the bean field mycoflora. Barley straw decomposition appears to present a substrate that is more favorable to the growth of certain saprophytic fungi, especially Chaetomium sp., than to the bean Fusarium. The saprophytic fungus in turn produces a diffusible, inhibitory principle which appears to have a three-fold effect on Fusarium: 1) it restricts vegetative growth in the soil following induced chlamydospore germination; 2) it induces sporulation and conidial formation, resulting in estimations of normal soil populations; and 3) it inhibits infestion of growing beans in some manner. Breakdown products of barley straw have a more direct effect on R. solani, which is either a suppressive effect on the saprophytic activity of the fungus or a stimulating effect on strongly competitive fungi such as Cephalosporium, Alternaria, and Cladosporium, resulting in reduced soil populations of the fungus. The incorporation of barley straw provides a favorable substrate for the saprophytic activity of T. basicola, which results in no apparent change in infectivity to beans but higher recoverable soil populations of the black root-rot fungus.

The results are consistent with those of Snyder, Schroth, and Christou (4) regarding the importance of the C:N ratio of the crop residue. The selective effects of barley straw amendment on bean root-rot pathogens and associated fungi makes the growing of a crop of barley and turning under the straw after the crop has matured, at which time the C:N ratio of the straw is high, appear a practical control measure for pinto bean root-rot, where T. basicola is not the major pathogen.

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FIGURE 1. Effect of barley straw amendment at 1% dry weight of soil on: A. pinto bean root-rot severity; and B. soil populations of pathogens, in naturally infested bean field soil.

FIGURE 2. Effect of green barley residue on the severity of pinto bean root-rot induced by Rhizoctonia solani, Fusarium solani f. phaseoli, and Thielaviopsis basicola in naturally infested and sterilized, reinfested field soil.

FIGURE 3. Effect of barley straw amendment on the severity of pinto bean root-rot induced by Rhizoctonia solani, Fusarium solani f. phaseoli, and Thielaviopsis basicola in naturally infested and sterilized, reinfested field soil.

FIGURE 4. Influence of barley straw amendment on populations of bean root-rot pathogens in both naturally infested and sterilized, reinfested field soil.

FIGURE 5. Influence of barley straw amendment to bean field soil on the populations of the 10 most commonly occurring non-pathogens in the bean rhizosphere. "Fusarium" includes both pathogenic and non-pathogenic Fusaria.

FIGURE 6. Effect of seeding barley straw amendment with an antagonistic Chaetomium isolate on the suppression of pinto bean root-rot by barley straw residue, A., and on soil populations of the pathogen, Fusarium solani f. phaseoli, B., through two bean plantings.

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TOBACCO ATTACKED BY A CYST-FORMING NEMATODE IN VIRGINIA

W. W. Osborne[1]

A cyst nematode belonging to the genus Heterodera has been found parasitizing Nicotiana tabacum variety Hicks in Amelia County, Virginia.

The known infested area consists of a single 3.3-acre field which has been planted to tobacco continuously for 7 years. The grower has observed stunted tobacco in certain areas of the field for the past 3 years. Cyst counts from these areas average 2040 cysts per pint of soil.

Symptoms expressed by infected plants are severe stunting (Fig. 1) and a greatly reduced root system (Fig. 2). Leaves of infected plants exhibit no abnormal discoloration; however, considerable wilting occurs during the warmer portion of the day. Examination of the roots reveals the presence of numerous dark brown oval cysts (Fig. 3).

This nematode closely resembles Heterodera tabacum Lownsbery & Lownsbery[2]; however, positive identification awaits the completion of morphological and host plant specialization studies. A survey is also being conducted to determine the geographic distribution and host range of this nematode in Virginia.

FIGURE 1. Stunted tobacco plants in the foreground are growing on soil heavily infested with the cyst nematode. Plants in the background growing on less heavily infested soil have matured to the blossom stage of growth.

[1]Associate Extension Plant Pathologist.

[2]Lownsbery, F. B., and J. W. Lownsbery. 1954. Heterodera tabacum new species, a parasite of solanaceous plants in Connecticut. Proc. Helminthol. Soc. Wash. D. C. 21:42-47.

FIGURE 2. Root systems of tobacco plants of the same age removed from cyst infested and cyst-free areas in the same field. Plant on the left possessed numerous cysts and showed a marked reduction in growth when compared with the cyst-free plant on the right.

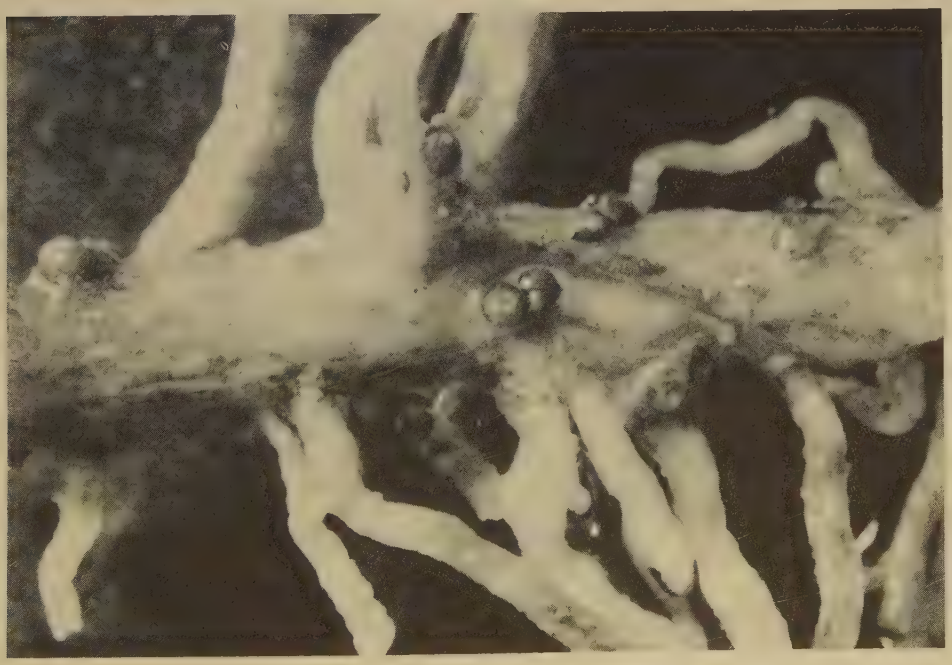

FIGURE 3. A section of tobacco root 1 cm in length showing numerous dark brown oval cysts.

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EFFECT OF VARIOUS HERBICIDES ON SOME SOIL FUNGI IN CULTURE[1]

Douglas C. Bain[2]

Summary

Several herbicides were tested for their effect on growth of Rhizoctonia sp., Sclerotium rolfsii, and S. bataticola in culture. Pentachlorophenol, 4,6-dinitro-o-sec-butylphenol, isopropyl N-phenylcarbamate, and 2-chloro-N,N-diallylacetamide inhibited growth of all three fungi for 24 hours or longer. Cyclohexanone, a solvent control, also inhibited growth. The amine salt of 4-(2,4-dichlorophenoxy) butyric acid, 4,6-dinitro-o-sec-butylphenol plus tris (2,4-dichlorophenoxyethyl) phosphite, and 3-(3,4-dichlorophenyl-1,1-dimethylurea, were effective against Rhizoctonia and S. rolfsii for 24 hours but not against S. bataticola. Pentachlorophenol and 2-chloro-N,N-diallylacetamide were considered to be the most effective materials against all three fungi. Rhizoctonia and S. rolfsii generally were more sensitive to the herbicides than S. bataticola.

INTRODUCTION

Spraying or dusting for control of diseases of forage and pasture crops is not considered practical; herbicides, however, are used on these crops either as pre- or post-emergence treatments, and their use appears to be on the increase. If some of the herbicides were fungicidal or fungistatic, it would seem that their use would tend to reduce losses caused by fungi--particularly on crops of biennial or perennial nature. Also, practicality of the use of herbicides would be enhanced. For over 25 years numerous papers concerning reaction of fungi to various growth-promoting chemicals have appeared in the literature. Many were reported to reduce growth of fungi and some even to inhibit growth. Some of these substances, such as 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), are used as herbicides. The literature concerning reaction of fungi to herbicides per se, however, is somewhat scant. Wilson (7) investigated the possibility of sodium pentachlorophenate (PCP) for control of Sclerotinia sclerotiorum (Lib.) d By. on apricots and almonds, and found that the material reduced sporodochia on twigs and old flower parts by as much as 87%. Results of work by Campbell (1) indicated that mint rust could be controlled by pre-emergence soil surface spray with "dinitro amine." Presumably, he referred to the amine salt of 4,6-dinitro o-sec-butylphenol (DNBP)-- an herbicide. Chappell and Miller (2) tested the effect of 18 herbicides at approximate field rates on Sclerotium rolfsii Sacc., Phytophthora parasitica Dast., var. nicotianae (Breda de Haan) Tucker, Helminthosporium victoriae Meehan & Murphy, Fusarium oxysporum Schlecht., f lycopersici Sacc., and F. conglutinans Woll. in culture. The fungi varied in their reaction but PCP and DNBP, at field rates, completely inhibited growth of all. PCP, DNBP, isopropyl N-(3-chlorophenyl) carbamate (CIPC), 2-chloroallyl diethyldithiocarbamate (CDEC), and 2-chloro-N,N-diallylacetamide (CDAA) completely inhibited growth of some of the fungi. In a field study of cultural practices for control of stem rot of peanuts, Garren (3) found that DNBP reduced infection by S. rolfsii about half as much as "non-dirting." He concluded that use of DNBP alone as a disease control measure was not justified. In a similar study, L. W. Boyle (personal communication) used sesone for control of peanut diseases but was unable to demonstrate it had a significant role as a fungicide. In one test, pod rot was "conclusively controlled by an overdose of sesone," but Boyle stated he was unable to repeat results of this test. McLean (6) treated soil infested with sclerotia of Sclerotinia sclerotiorum in an effort to inhibit development of apothecia. Among other materials, he used DNBP, PCP, diuron, and monuron. Results of some tests were not conclusive because the soil dried, and no results were given for PCP and diuron in other tests. Le Tourneau and Buer (5) found that PCP, 2,4-D, and 4-(2,4-dichlorophenoxy) butyric acid (4-(2,4-DB)) inhibited growth of Verticillium albo-atrum Reinke & Berth. about 50% in liquid synthetic medium. Further investigation of the effect of herbicidal materials on different fungi in culture and diseases they cause in the field seems desirable. This paper reports results of tests to determine the effect of a number of these chemicals on growth of Rhizoctonia sp., Sclerotium rolfsii, and S. bataticola Taub. in culture.

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The help of Tildon Easley concerning use of herbicides is gratefully acknowledged.

MATERIALS AND METHODS

Herbicides selected for the tests (Table 1) are, or possibly could be, used as pre- or post-emergence treatments for weed control in forage or pasture crops. Twelve other herbicidal materials (not listed in Table 1) were used in replicated tests with Rhizoctonia but were completely ineffective and thus were not tested against S. rolfsii and S. bataticola. Since recommended rates or concentrations of herbicides are often based on soil type, dilutions of the materials used were between high and low rates in pound/acre of active ingredients in water at the equivalent of 40 gallons per acre. Mercuric chloride (1-1000) was selected as a standard fungicidal control and dry filter paper discs (which became wet when placed on agar) as the non-fungicidal control. Although cyclohexanone is not frequently used as a solvent, it was for PCP in these tests and as a solvent check. Sterilized filter paper discs 6 mm in diameter were dipped momentarily in the chemicals, drained, and immediately transferred to potato-dextrose agar in disposable Petri dishes.

Two methods were employed for testing reaction of the fungi to the chemicals. In Method I, treated filter paper discs were placed on solidified agar in Petri dishes. Immediately thereafter, discs 6 mm in diameter and 1 to 2 mm thick cut from fungi actively growing on PDA were inverted and placed on the paper discs. Results of exploratory tests with $HgCl_2$ indicated that fungus growth was invariably inhibited if the inoculum discs were inverted, but when placed top-side-up colonies of the fungus occasionally grew out onto the agar. Four paper discs and four

Table 1. Herbicides used in tests with Rhizoctonia sp., Sclerotium rolfsii, and S. bataticola, and rate in pound/acre of active ingredient.

Common name[a]	Chemical name of active ingredient	Rate pound/acre active ingredient
4-(2,4-DB)	4-(2,4-dichlorophenoxy) butyric acid (amine salt)	2
DNBP	4,6-dinitro-o-sec-butylphenol	3
DNBP + Falone	+ tris (2,4-dichlorophenoxyethyl) phosphite	3:1
IPC	isopropyl N-phenylcarbamate	8
Diuron	3-(3,4-dichlorophenyl)-1,1-dimethylurea	2
PCP	pentachlorophenol	6
CIPC	isopropyl N-(3-chlorophenyl) carbamate	8
CDEC	2-chloroallyl diethyldithiocarbamate	8
CDAA	2-chloro-N,N-diallylacetamide	8
Stauffer 1607	N-propyl-di-n-propylthiocarbamate	6
Cyclohexanone	diluent for PCP	

[a] Approved by the Weed Society of America.

Table 2. Mean diameter of fungus colonies in cm, including 0.6 cm for diameter of inoculum disc, 24 and 72 hours after plating. Values are the mean of three tests, two plates per treatment per test, four discs per plate.

	Rhizoctonia sp.		S. rolfsii		S. bataticola	
Treatment	24 hours	72 hours	24 hours	168 hours	24 hours	72 hours
4-(2,4-DB)	0.60	0.60	0.60	0.80	0.61	2.13
Dinitro	0.60	0.76	0.60	0.60	0.60	1.50
Dinitro + Falone	0.60	0.60	0.60	0.60	0.60	0.80
IPC	0.60	0.93	0.60	1.03	0.60	1.33
Diuron	0.60	1.03	0.60	0.70	0.96	3.20
PCP	0.60	0.60	0.60	0.60	0.60	0.60
CIPC	0.61	1.26	0.60	0.90	0.60	2.06
CDEC	0.76	2.26	0.76	3.20	0.95	3.20
CDAA	0.60	0.60	0.60	1.46	0.60	0.93
Stauffer 1607	0.85	1.33	0.60	2.76	0.60	2.70
Cyclohexanone	0.60	1.00	0.60	0.60	0.60	0.93
Check - water	1.40	3.20	2.18	3.20	1.96	3.20
Check - $HgCl_2$	0.60	0.60	0.60	0.60	0.60	0.60

Table 3. Zone of inhibition in cm, including 0.60 cm for diameter of filter paper disc. Kulik and Tims method; mean of three tests with three plates per test and three discs per plate. Notes taken at about 24 hours after plating except S. rolfsii, which was about 48 hours.

Treatment	Rhizoctonia sp.	S. rolfsii	S. bataticola
4-(2,4-DB)	1.81	1.80	0.66
DNBP	1.36	2.32	0.81
DNBP + Falone	1.97	2.41	0.72
IPC	1.12	1.21	0.77
Diuron	1.26	2.09	0.60
PCP	4.00[a]	4.00[a]	1.68
CIPC	1.25	0.93	0.62
CDEC	0.60	0.60	0.60
CDAA	2.87	1.31	1.61
Stauffer 1607	0.68	0.64	0.60
Cyclohexanone	0.73	1.03	0.83
Check - water	0.60	0.60	0.60
Check - $HgCl_2$	2.27	1.66	2.24

[a] Fungus growth inhibited throughout Petri dishes.

inoculum discs were used per test. Inhibition of growth indicated fungicidal or fungistatic activity of the chemicals tested. Lactic acid was added to PDA in test plates of 4-(2,4-DB), diuron, CIPC, CDEC, and Stauffer 1607. Shortly after dilutions of these were made up they became contaminated with bacteria. Later tests, however, indicated the bacteria did not materially affect growth of the fungi nor did the acid affect activity of the herbicides.

The other method was essentially the same as that employed by Kulik and Tims (4). Potato-dextrose liquid medium, 200 ml per flask, was seeded with 3 to 6 mm discs of actively growing fungus. After about a week or 10 days, contents of the flasks were blended in a Waring Blendor for 1 minute. Twenty-five ml of the blend plus two drops of lactic acid were added to 100 ml of melted and cooled PDA. Roughly 15 ml of this mixture were poured per plate. Shortly after the agar solidified, three treated paper discs were spaced on the agar. Three plates were used per test. These were incubated at room temperature and readings were taken 1, 2, 3, and 7 days after seeding. Diameter (including 6 mm of paper disc) of the zone of inhibition around the paper discs in the Kulik and Tims method indicated activity of the chemicals. Each material was tested three or more times by both methods.

RESULTS AND CONCLUSION

The mean diameters of fungus colonies that grew out from inoculum discs are given in Table 2. In general, fungi seeded on paper discs moistened with water formed colonies 1 cm or more in diameter in a 24-hour period while those seeded on $HgCl_2$ did not grow at all. None of the fungi grew out from inoculum discs on paper treated with 4-(2,4-DB), DNBP, DNBP + Falone, IPC, diuron PCP, CIPC, CDAA, and cyclohexanone within 24 hours. The diameter of Rhizoctonia colonies on Stauffer 1607 represents the mean of two small colonies, so that for all practical purposes this chemical also inhibited growth of all three fungi. Growth of Rhizoctonia in the first 24 hours was also inhibited (except for one or two small colonies each) by EPTC, dalapon plus 4-(2,4-DB), and CDEC but was not affected by the other materials; however, these herbicides were not considered effective after 48 hours. About 72 hours after seeding, the 10 chemicals listed above were still effective against Rhizoctonia, and even at 168 hours against S. rolfsii (except Stauffer 1607); however, only DNBP, DNBP + Falone, IPC, PCP, CDAA, and cyclohexanone were still effective against S. bataticola. At the 72-hour reading, where diameters were 1.5 cm or less, only two or three colonies had grown from the 24 seedings.

Results obtained in the Kulik and Tims Method were considered to give a more accurate picture of the fungicidal or fungistatic properties of the herbicides. Data in Table 3 represent the mean diameters (including 6 mm for the paper disc) of the zones of inhibition. With the exception of CDEC, Stauffer 1607, and cyclohexanone, all materials were effective against Rhizoctonia. All except CIPC, CDEC, and Stauffer 1607 were effective against S. rolfsii; however, only DNBP, IPC, PCP, CDAA, and cyclohexanone were effective against S. bataticola. By this method, CDEC and Stauffer 1607 were the only herbicides that were ineffective against all three

fungi. Excluding PCP, S. bataticola and Rhizoctonia were definitely most sensitive to CDAA but S. rolfsii was most sensitive to DNBP, DNBP plus Falone, and diuron. Forty-eight hours after plating, the fungi had invaded the original zones of inhibition. Rate of invasion varied somewhat with treatment, for example, Rhizoctonia invaded the $HgCl_2$ zone rather rapidly but had hardly entered CDAA and 4-(2,4-DB) zones. By 72 hours Rhizoctonia had not grown up to discs treated with 4-(2,4-DB), DNBP, DNBP plus Falone, IPC, PCP, and CDAA, but had overgrown the $HgCl_2$ discs. Aerial growth of Rhizoctonia throughout plates was so reduced by CDAA that the actual zones of inhibition were somewhat masked and did not show up clearly. S. rolfsii also had not overgrown discs treated with the above-listed materials as well as diuron and CIPC even though CIPC had only a narrow zone of inhibition at 48 hours. Growth of Rhizoctonia and S. rolfsii in any part of the Petri plates was almost completely inhibited by PCP. S. bataticola had overgrown all treated discs except those dipped in PCP. Sclerotial production by this fungus in most treatments was limited (at 72 hours) to margins of original zones of inhibition.

Inhibition of growth of Rhizoctonia sp., S. rolfsii, and S. bataticola in culture by certain herbicidal materials under the conditions described has been demonstrated. Preliminary results of greenhouse tests in which these herbicides are being used with Ladino clover, Rhizoctonia sp., and S. rolfsii substantiate in part those reported herein. Pre- or post-emergence (or both) applications of these herbicides to forage and pasture, and indeed vegetable crops should tend to reduce losses caused by these fungi, particularly where chemical weed control is regularly practiced.

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CONTROL OF BOTRYTIS ROT OF STRAWBERRIES

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Summary

Gray mold, caused by Botrytis cinerea, was the most important fruit rot of Louisiana strawberries. A study was undertaken for the control of this disease in which many fungicides, including organic and inorganic compounds and antibiotics, were first screened in the laboratory, the most promising of which were later field tested. Of these Phaltan, Dyrene, dichlone, thiram, DHA-S, and captan were the most effective materials tested. Captan was considered the best fungicide for commercial use because of its low mammalian toxicity in addition to its effectiveness in control of B. cinerea and Mycosphaerella fragariae. Thiram was recommended for control of fruit rot only. All varieties of berries tested were susceptible to B. cinerea determined by the "fruit dip" method of inoculation in the laboratory.

INTRODUCTION

Many workers in the United States and other countries (1-20) have contributed to the chemical control program of strawberry fruit rot caused by B. cinerea Pers. ex Fr. However, because Louisiana's method of strawberry culture is unlike that of any other State in that strawberries in Louisiana are grown as annuals and because the rot can become extremely severe here, it was important to find a control for the disease under Louisiana conditions.

METHOD OF SCREENING AND EVALUATING FUNGICIDES

In an intensive program to find a control for gray mold, fungicides which included organic and inorganic chemicals and antibiotics were screened in the laboratory using two methods. One was the conventional slide method where fungicides were sprayed on microscope slides, allowed to dry, then later spore suspensions (water + 1% yeast extract + 2% dextrose) of the fungus were added to the slides and placed in high humidity chambers at room temperatures. The percentage germination was recorded 24 to 72 hours later. The other "fruit dip" method of screening fungicides was described in a previous publication (5). After a fungicide was laboratory tested and found to be fungicidal to B. cinerea as well as suitable for field application, it was used in field trials to test further its effectiveness.

Because the fungus required prolonged periods of high humidity to produce enough rotted berries to enable evaluation of the materials tested, a specially constructed series of nozzles that sprayed water continuously around the plants was used (Fig. 1). This system, with a little assistance from natural humidity, provided fairly good conditions for development of the disease. Forty-five treatments not including the controls were tested.

Field plots were 32 feet long and contained 50 plants each in the 1957, 1959, and 1960 tests. They were 95 feet long in the 1961 tests and contained 150 plants. All were set on 42-inch rows. The plants were grown using the same cultural practices that the growers used.

The fungicide spray programs were started when blossoms began to appear, usually sometime during February. The materials were applied with a 50-gallon John Bean Sprayer using about 200 pounds' pressure with the spray directed to get good coverage under the plants as well as on top.

The fungicides were used at various rates depending on the material in a 7 to 10 day spray schedule. Some fungicides were more phytotoxic than others so that there were variations in the concentrations used, 1/2 to 4 pounds per acre.

The chemicals used were: sodium salt of dehydroacetic acid (Harven, DHA-S), alpha, alpha trithiobis (N-dimethylthio formamide) (Union Carbide Experimental Fungicide 5400), 2,4-dichloro-6-(o-chloroanilino)-s-triazine (Dyrene), alkylisoquinolinium chloride (Ammonyl 781), captan, N-trichloromethyl thiophthalimide (Phaltan), thiram, cholomine + potassium iodide (Heliogen), bis-3(3,4-dichloro-2(5)-furanonyl)ether (General Chemical Experimental Fungicide 2466), alkyldimethylbenzylammonium chloride (BTC-824, BTC-1100, BTC), phenylmercuriedimethyldithiocarbamate (Merbam), N-phenylmercuriethylenediamine (Coromerc), dichlone,

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FIGURE 1. Method used to help induce rot in the field. Note the 15-inch risers that support the nozzles that direct the water spray parallel to the ground.

Merk HMF proprionate, neomycin sulfate, and sorbic acid (Sorbistat).

ANALYSIS OF FIELD FUNGICIDES

The effect of the fungicides on the control of the organism is shown in Table 1. These data represent the rots that occurred during the period of greatest infection which extended from the latter part of April to the middle of May and not for the entire season.

The rot was somewhat more severe in 1957 than in 1959, 1960 or 1961. Table 2 indicates the amount of rot that occurred under good conditions for the development of the fungus, and the average yields in crates per acre. The yield data shown were not for the entire season, but were recorded during a consecutive number of days on which rots were most prevalent. The yields in general reflected the percentage rot that occurred in any one treatment, so that when the percentage rot was high, the yields were low and vice versa. This was particularly true in the case of the Konvoy variety.

To demonstrate briefly the effect of the rot on yields, however, earlier tests with the Klondike variety showed that captan, Fungicide 5400, and DHA-S treated plants during the course of the entire season produced higher yields than untreated plants and when the yields were high the rot was low, and vice versa (Table 3).

FARMER COOPERATIVE FUNGICIDE TESTS

Candidates considered effective enough against the organism to become possible recommended materials chosen by the field screening procedure were used in field tests in commercial plantings. These were thiram, captan, ziram, DHA-S, and Fungicide 5400, all of which were discontinued in later tests except for thiram and captan. During the course of these experiments rot became a problem on several occasions in which captan treated plants produced significantly higher yields than nontreated plants. Plots ranged from 1500 to 3000 plants, some single and other double set, which were sprayed in a 7- to 10-day schedule that began the first of March. Harvests were made and the data were recorded by the growers. The numbers of rotted berries were not recorded, thus data were of yields of marketable berries which were taken usually during the periods of greatest rot. It was believed that the yields of ber-

Table 1. Mean percentage rot of Konvoy and Headliner berries from plants sprayed with various fungicides, 1959, 1960, 1961.

Treatment	Variety and year				
	Konvoy		Headliner		
	1959	1960	1959	1960	1961
Captan (control)	9.0	9.5	3.7	5.2	1.6
Dyrene	12.5	11.4	9.2	7.6	--
Ammonyl 781	--	18.1	--	15.8	--
Phaltan	--	8.6	--	5.3	--
Thiram	10.9	14.9	2.5	8.4	2.0
Heliogen	29.0	--	22.3	--	--
2466	14.1	--	10.5	--	--
BTC-824	28.9	--	21.7	--	--
Harven	--	16.6	--	11.2	--
Sorbistat	--	--	--	--	8.5
Sodium proprionate	--	--	--	--	12.8
Neomycin	--	--	--	--	16.0
BTC-1100	--	--	--	--	14.4
LSD 5%	11.5	3.1	7.0	3.3	6.6
1%	15.9	4.3	9.6	4.5	9.1

Table 2. Mean percentage rot of fruit and yields in crates per acre of the Headliner and Konvoy varieties sprayed with different fungicides in the field, 1957.

Treatment	Mean % rot		Mean yields	
	Headliner	Konvoy	Headliner	Konvoy
Captan + BTC	10.0	35.0	35.2	21.2
Merbam	25.0	71.0	37.1	20.1
Captan (control)	7.3	22.0	47.3	30.8
Thiram	7.9	21.0	44.8	28.5
Coromerc	6.6	34.0	40.0	21.0
BTC	38.6	86.0	32.2	15.8
LSD 5%	10.6	42.7	11.1	5.7
1%	14.4	58.7	18.2	7.8

Table 3. Mean yields of berries and percentage rot for the entire season from treated and non-treated field-grown Klondike plants.

Treatment	Yields in crates/acre	% Rot
DHA-S	301	14.1
5400	348	12.9
Captan	441	9.9
Control	251	26.5

Table 4. Numbers of berries from various seedlings and varieties rotted by B. cinerea in the laboratory "fruit dip" method[a].

Variety or seedling number	Numbers of berries rotted[b] (days after inoculation)					
	1	2	3	4	5	6
Klondike control	0	0	0	1	2	6
Klonmore control	0	2	5	5	9	12
Klondike	0	11	13	16	21	25
Klonmore	0	16	20	24	27	29
Konvoy	0	10	16	24	25	25
Marion Bell	0	11	20	22	24	26
Headliner	0	6	11	17	18	26
0-161	0	4	8	11	14	19
742	0	7	11	14	15	18
0-242	0	13	18	18	19	21
0-187	0	13	24	25	26	29
0-162	0	9	15	17	23	24
L-27	0	4	11	17	19	23
Tuzzalino Bell	0	10	15	23	--	--
Tuzzalino Beauty	0	12	17	22	--	--

[a]A total of 30 berries were tested for each variety or seedling from three different tests.
[b]The number of rotted berries was totaled on each day so that the numbers in each column are accumu-

ries reflected the amount of rot in the various plots, that is, when rot was high yields were low and vice versa. This was particularly true in the case of captan treated plants versus nontreated plants.

VARIETAL SUSCEPTIBILITY

Eleven lines of strawberries were tested in the laboratory for resistance by the "fruit dip" method. Controls were fruit not dipped in the conidial suspensions and placed in similar containers. The numbers of berries that rotted during the 6-day period were recorded; totals of three tests are given in Table 4. All lines were considered susceptible although some appeared somewhat less susceptible than others. Nevertheless, the fungus was able to infect readily any of the commercially grown berries listed here.

DISCUSSION

It was not necessary to list all the fungicides tested in this investigation. However, the materials that were used in field trials from 1951-1961 that were considered most suitable for field application are discussed briefly.

Thiram, according to Stoddard and Miller (19), gave better control of gray mold than did captan and for long periods after spray application. The tests at the Louisiana State University Experiment Station indicated no significant difference between them in rot control. Thiram, however, has a lower tolerance and cannot be applied to strawberry fruit less than 3 days before harvest, whereas captan can be used at any time during the harvest period, a big advantage of captan over thiram. Under Louisiana conditions berries are frequently harvested from the same plants every other day for fresh market. In addition, captan controls leaf spot caused by Mycosphaerella fagariae (Tul.) Lindau. Although thiram was not evaluated for this property at the Louisiana State University Experiment Station, Cox and Winfree (2) reported that it was least effective of the fungicides used in field tests for the control of leaf spot. Because the leading strawberry variety is susceptible to leaf spot, certainly a choice of fungicide would be important here. On the other hand, Parry and Wood (13, 14) were able to build up a resistant strain of B. cinerea to captan which they were unable to do with thiram. Both captan and thiram, however, were recommended for Botrytis rot control in Louisiana.

The dimethyl group of the carbamates were weakly fungicidal to the fungus whereas the ethylene group did not survive the laboratory screening stage. Ziram showed some control. Cox and Winfree (2) indicated that repeated applications of nabam + zinc sulfate or zineb appeared to increase Botrytis gray mold. No such indications were noted in the work presented here. Stoddard and Miller (19), although their tests were carried out with plants in the greenhouse, showed that nabam and ziram gave poor control of the disease.

Dichlone and Dyrene controlled gray mold as well as captan, but again the fact that captan has a much greater tolerance eliminated these two candidates temporarily. Both dichlone and Dyrene burned the foliage at the higher concentrations used. Lowering the concentration to a safe level, however, did not reduce the effectiveness of control. Cox and Winfree (2) reported that these materials caused leaf damage in the form of bronzing and some stunting.

DHA-S appeared to be a promising material in the field. It has a high tolerance and was rated high in the first field screening test. However, it caused a brown discoloration on the light exposed surface of the berries and sometimes produced a leaf chlorosis. When DHA-S was used at the start of these investigations, the spray was made from the technical material which might have had some effect on phototoxicity. The same fungicide, formulated now as Harven, showed no toxicity at the lower concentrations. Nevertheless, in field tests Harven did not give as good control as captan.

Phaltan was as effective in control as captan. But again the difference in tolerance between the two eliminated Phaltan during the field plot trials.

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CROWN GALL DISEASE IN ISRAEL[1]

Zafrira Volcani

Crown gall disease on fruit trees, and on other plants of various families, has been observed in Israel for many years. The disease causes severe damage in nurseries and in stone fruit orchards, with the highest rate on trees grafted on almond rootstock. The extension of orchards and vineyards throughout the country during the last 12 years has aggravated this problem, particularly with respect to stone fruit trees.

This disease was first described on deciduous fruit trees, in Israel, by Perlberger in 1939 (4). During the past decade Papo (2, 3) continued studies on the disease, based mainly on a survey of disease occurrence and incidence and on preliminary trials. No basic investigations of the disease or its causal agent and its effect on different plants, as well as its host range, have been made. Indeed its causal agent was never isolated or identified by Perlberger or Papo. An investigation was therefore undertaken into these problems. In June 1960 a bacterial organism was isolated for the first time in pure culture by the author from roots and collar tumors of almond rootstock. This organism produced galls when inoculated first on Kalanchoë roots and stems (Fig. 1), which served as a test plant to detect the organism, and later on stems of tomato[2], sunflower, almond and apricot rootstock. (Fig. 2).

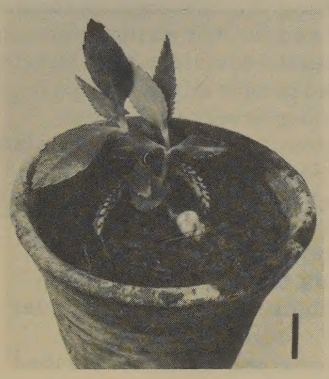

FIGURE 1. Inoculated Kalanchoë plant, with an almond isolate of A. tumefaciens, showing the appearance of a gall at the base of the stem near the collar, around inoculation wound.

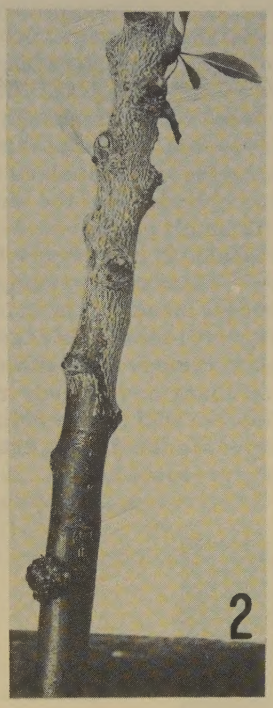

FIGURE 2. Inoculated almond rootstock, with the same culture of A. tumefaciens, showing the appearance of a gall on the stem, near inoculation wound.

Different types of colonies were obtained with organism on nutrient agar and nutrient agar + 1% glycerol. A smooth type, which often became rough in older cultures, appeared on the former medium, while a shining jelly-like type, which later became smooth, was predominant on the medium with the carbohydrate. Lower temperatures of 14° to 20° C were more favorable for the development of the latter growth than higher temperatures of 25° to 30°. No difference in effect was observed between the two types, derived from the same isolate, in the results of inoculation tests on Kalanchoë plants.

Examination of the organism and its reisolations revealed that it is closely related morphologically, physiologically and pathologically with the crown gall organism. It was thus identified as Agrobacterium tumefaciens (E. F. Sm. & Town.) Conn (1).

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[1]Publication of the National and University Institute, Rehovot. 1961 Series, No. 398 E.
[2]Inoculation tests on tomato, sunflower, almond, and apricot rootstock were made by D. Zutra, a student at the Faculty of Agriculture.

A GLASS AND CELLULOSE ACETATE INSECT CAGE[1,2]

D. B. Protacio

Insect cages of convenient size and having some advantages over those previously described by Costa and Bennett (3), Adsuar (1), Giddings (4) and Maramorosch (5) were developed recently for use in aphid transmission experiments.

The cages shown in Figure 1 were made from printing press discards of transparent cellulose acetate[3] material.

Rectangular sheets of the desired size were cut. A vial, coffee jar, beaker or any cylindrical glass may be used as a base for making the cages by simply rolling the rectangular sheets of cellulose acetate around the chosen cylinders (bottom removed with red hot wire ring technique) until the two ends overlap and then gluing with acetone along the cut edge. Another sheet can then be placed over the first and fastened in the same way. Two telescopically joined cylinders are thus formed. The outer cylinder must be loose enough to move freely in any desired direction but tight enough not to slip accidentally. To cover the top, the outer cylinder is moved slightly beyond the inner (to avoid gluing the two cylinders together), inverted over a square sheet as wide as the mouth of the outer cylinder and the sheet glued on with acetone.

When the "coffee jar cages" are used with small plants, in 2- to 4-leaf stages, the glass jar should be slightly pushed into the soil to render it insect-proof. Holes can be cut with a cork-borer in each cellulose acetate cylinder. When the matching holes coincide, the cage is in an open position and insects may be introduced through this hole. Rotating the outer cylinder in any direction or simply disaligning the holes will close the cage.

The advantage of the "coffee jar cage" over the usual muslin cages as previously described (2) is that the insects are given a very limited space and in their movements almost always have contact with the plants. In addition, the coffee jar will not be deformed and the cage will admit more light than some cages. Furthermore, putting the pots in trays of water may not be necessary, since migration of insects is completely prevented.

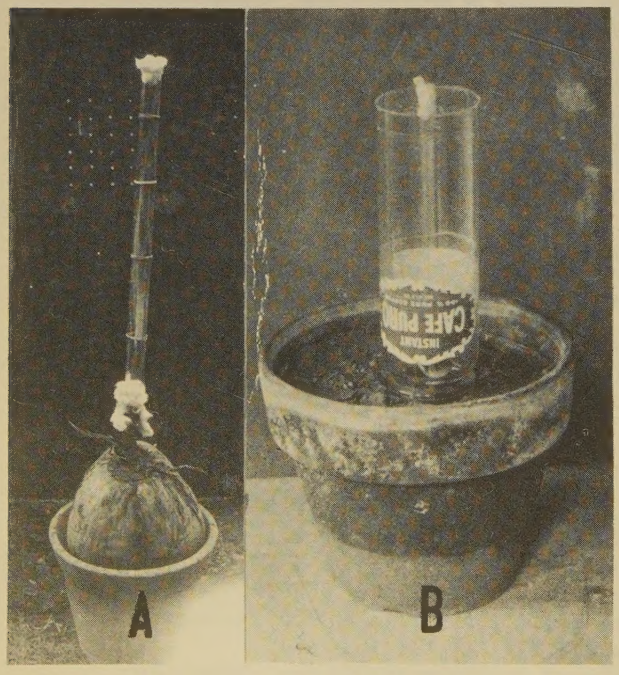

FIGURE 1. Two types of cages. A -- Cellulose acetate tube using paper clips and rubber band as binder. B -- Coffee jar (bottom previously removed) with a cellulose acetate sheet fitted on it. (Photo by courtesy of J. Nuñez.)

[1]Published with permission of Mr. Eugenio E. Cruz, Director, Bureau of Plant Industry, Department of Agriculture, Republic of the Philippines.

[2]Grateful acknowledgment is made to Dr. K. Maramorosch of the Boyce Thompson Institute for Plant Research and to Dr. F. O. Holmes of the FAO for their help in the preparation of this paper.

[3]Thanks are due Mr. L. Nuñez and colleagues of the Manila Times Publishing Co. for supplying the material.

The use of the cellulose acetate material has its limitations too. Some cellulose acetate is toxic to plants and to offset this Maramorosch (5) suggested that cellulose nitrate may be used instead, although this material may constitute an appreciable fire hazard.

Furthermore, the use of cotton wool and glass (coffee jar) to prevent direct contact of cellulose acetate with the plants during short exposure periods prevented bad effects on tomato, tobacco and coconuts.

The simplest tubes to make are those shown in Figure 1A for which rectangular celluloid sheets were rolled and the overlapping ends on top and bottom were held together by ordinary paper clips. The edge was not glued. Two or more loops of ordinary rubber bands were rolled down at convenient spacings to keep the sheets in a tube-like form. Cotton wool was used to wrap the base of the coconut seedlings and to hold the tubes in place. Another cotton plug was used at the top end. As in the coffee jar cage, holes were cut with a cork borer at the sides (the exact location of holes will depend on the operator's convenience) and then plugged with cotton to prevent the ingress and escape of insects.

The "coffee jar cage" and tubes made out of non-toxic, acid resistant material may be useful to plant pathologists engaged in inoculation with spore suspensions of plant pathogens.

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A POWDERY MILDEW ON SAFFLOWER[1]

Bill B. Berkenkamp[2]

A powdery mildew (Oidium sp.) appeared on greenhouse plantings of safflower, Carthamus tinctorius in December 1960. The safflower was being grown in aerated liquid, and sand culture supplied with nutrient solutions. The greenhouse temperature ranged between 55° and 74° F. The powdery mildew was plentiful on lower leaves and stems, particularly on senescent leaves, and conidia were produced abundantly. The infected plants were periodically examined for cleistothecia, but none were found. Attempts to transfer the powdery mildew to sunflower failed. Transfers to young safflower leaves were rarely successful and the organism was lost. To the author's knowledge, this is the first report of a powdery mildew on safflower in the United States.

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NEMATODE ON TABEBUIA PENTAPHYLLA IN EL SALVADOR

Oscar Ancalmo[1]

Root-knot nematodes on Tabebuia pentaphylla, the national tree of El Salvador, were recorded for the first time in the country in 1958[2].

In 1960 a preliminary survey in two government nurseries, one at Santa Tecla and the other at San Andrés, both in the Departmento of La Libertad, showed Meloidogyne incognita acrita Chitwood[3] attacking seedlings of Tabebuia.

Poor growth and severe galling of both the tap root and secondary roots was noted. Aerial symptoms of the seedlings attacked by this species of Meloidogyne included foliar chlorosis, necrosis and stunting.

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[1]Plant Pathology Department, Dirección General de Investigaciones Agronómicas, Santa Tecla, El Salvador.

[2]Ancalmo, Oscar. 1959. Lista Preliminar de Enfermedades Parasitarias En las Plantas de El Salvador. Boletín Técnico #22, Ministerio de Agricultura, Centro Nacional de Agronomía.

[3]Identification of the species kindly made by Dr. R. P. Esser.

AERIAL SURVEY FOR SUGAR BEET NEMATODE

Abraham H. Epstein

Symptom surveys for the sugar beet nematode, Heterodera schachtii, may be greatly facilitated by the use of light aircraft. They have been especially helpful in Wisconsin where the sugar beet acreage is scattered over 15 counties and many of the plantings consist of 10 acres or less.

The symptoms of sugar beet nematode infestations visible to the airborne observer are irregular patches of yellowed, stunted sugar beets. In Wisconsin these symptoms are usually most pronounced during the first 3 weeks of September, following the high temperatures prevalent during the latter part of August.

The locations of the sugar beet plantings are obtained from the local sugar company fieldmen and these are then plotted on county highway maps (scale of 1/2 inch to the mile) according to the appropriate township, section, and quarter section if possible. It is not difficult to find these locations from the air because of the very distinctive shade of green characteristic of sugar beet plantings.

Each field is observed from an altitude of 500 feet. The aircraft should pass over that side of the field which will place the observer looking away from the sun. The positions of any suspect portions of the field, as well as any pertinent directions for locating it on the ground, are noted by the observer. The surveys are best carried out between the hours of 9:00 a.m. to 2:00 p.m. on days when glare and haze conditions are at a minimum. Calm air with some high broken cloud cover provides ideal conditions for this work. Suspect fields located in this manner are subsequently checked on the ground and soil samples collected for the laboratory.

A number of different types of aircraft may be used; however, the most efficient for such close work appears to be the single-engined craft with tandem seating arrangement. Air speed while observing the field should be under 90 miles per hour.

Surveys utilizing this method reduced the time and manpower requirements to approximately half that required for conventional ground inspection.

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